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THE GENETICAL EFFECTS OF MUSTARD GAS SUBSTANCES AND NEUTRONS

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INTRODUCTION.

SINCE MULLER's fundamental discovery in 1927 that X-rays increase the mutation rate immensely, artificial means of changing genes and chromosomes have become a valuable tool in genetical research work. The ClB-method designed by him allowed of an appropriate and rapid determination of recessive sex-linked lethals in *Drosophila*. In experiments applying neutrons and chemical substances as mutagenic agents lethals were likewise almost exclusively used as test mutations. In the case of neutron research there is, as far as we know, no single study of visible and viable mutations.

The Svalöf work on induced mutations centers around the production of viable or, rather, vital (high-productive) mutants in agricultural plant species. From many points of view these species are ideal objects for evolutionary discussions. The mutants, whether they have arisen spontaneously or artificially, can be appropriately tested in what may be called their »natural habitat», i. e. arable land. The agricultural and, then, the evolutionary fitness of a mutant can be measured by exact methods, considering on one hand practically important properties like yield, earliness, stiffness of straw, seed quality, on the other hand properties like number of seeds produced per plant, or competitive ability determined on vegetative characters.

The chlorophyll mutations, frequent in diploid plant species, correspond to the sex-linked recessive lethals of *Drosophila* in many ways. They are especially valuable as test mutations in experiments concerning dosages, pre-treatments, etc. In addition, however, the chlorophyll mutations function as »visibles», since a great number of different types can be distinguished. The application of neutrons and mustard gas substances is to be regarded as a link in the Svalöf experiments dealing with controlled mutability, as these agents were previously known to act on genes and chromosomes in much a different way from that of X-rays. The results to be reported here form a preliminary account of invest-

igations, which are being further continued by the junior author. Co-operation with Prof. Dr. M. SIEGBAHN (The Research Institute of Physics, Stockholm) and Prof. Dr. G. LJUNGGREN (The Research Institute of National Defense, Stockholm) has made these experiments possible. In addition, we are indebted to Mr. A. HEDGRAN for his unfailing interest in the matter of the neutron experiments, as well as to Dr. A. FORSSBERG for his valuable help with regard to the X-ray treatment.

MATERIAL AND METHODS.

Since neutrons and mustard gas substances have not previously been applied in cereal species, special technics had to be elaborated. A brief report on this point will be given here. A more comprehensive account will be published when the technics have become definite and fully accurate.

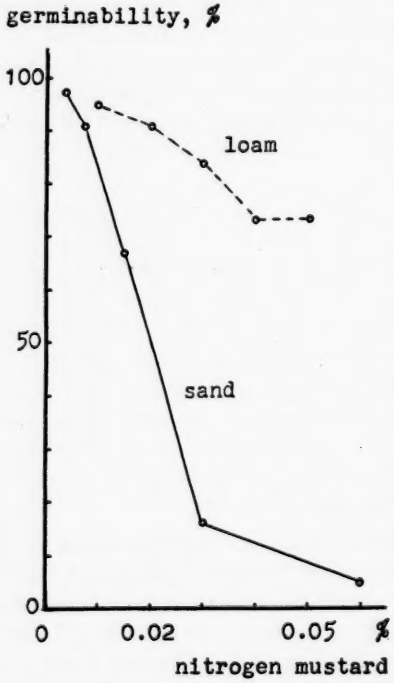

Fig. 1. The different germinability in laboratory (sand) and field (loam) cultures after treatment with nitrogen mustard.

Mustard gas substances. — The water-soluble nitrogen mustards are suitable agents for seed treatments. They permit of an exact time of exposure. Furthermore, the experiments are entirely reproducible if fresh solutions are used for a relatively short time and under exact temperatures. In the present study we concentrated on a special nitrogen mustard, dichlorodiethylmethylaminhydrochloride, $HCl \cdot CH_3N(CH_2CH_2Cl)_2$. With the usual sulphur mustard only preliminary experiments were carried out.

The nitrogen mustard used is easily hydrolysed (with a temperature of 20 centigrades about 30 % of the salt is hydrolysed in 40 minutes). We therefore chose the concentration as the variable factor. The time of exposure (40 minutes), the quantity of solution (150 cc in a closed Erlenmeyer flask of 300 cc), the temperature (21—22 centigrades), the intensity of shaking the flasks and the number of seeds treated (300 seeds) were kept constant. At the end of the treatment the seeds were washed in tap water for ten minutes, wiped off between filter papers and immediately sown.

The germination of the first experimental series was determined indoors in sand. By this method the salt concentrations suitable for field experiments were

estimated as 0,01—0,05 per cent. However, in spite of more unfavourable conditions for germination the field experiments showed less effect from the nitrogen mustard than the indoor experiments (Fig. 1). The same experience was gained with regard to the common mustard gas. There are at least two alternative explanations possible. Either the cause is associated with the high content of absorbing humus substances in loam soil in comparison with sand, or with the different conditions (water, temperature, etc.) influencing the onset of germination and preventing the full effect of the mustard gas substances. Whatever the cause, the field series were treated insufficiently.

When treatment was made with sulphur mustard gas, about two hours of exposure in saturated vapour appeared to be critical. There was, however, a great difficulty in obtaining exact dosages; the reproductivity of the treatment was unsatisfactory. Thus, in three independent experiments the germination capacity after two hours of exposure was 1, 35 and 42 % respectively. The technics of treatment must therefore be further elaborated before exact data of the mutagenic ability of mustard gas can be presented for cereal species.

Neutrons. — The neutrons of the present investigation were obtained from the small cyclotron of the Research Institute for Physics in Stockholm, an equipment recently described by ATTERLING (1945). The deuteron energy of the cyclotron is 6,7—7 MeV. In order to ensure a stable run the neutron beam from the beryllium target was regulated to give an ionization current of $0{,}6 \cdot 10^{-7}$ A in a boron chamber. The fast neutrons produced were inhomogeneous, having energies up to circa 8,5 MeV. The seed was placed in glass tubes (with an outside diameter of 15,5 mm and a thickness of 0,8 mm). The glass tubes were circularly arranged in a fixed wooden plate between the magnet poles but outside the vacuum chamber and at a distance of 20 cm from the target. In order to exclude as much gamma radiation as ever possible the tubes were covered with a Pb plate 2 mm in thickness. The time of exposure ranged from 15 up to 75 minutes.

The dosimetry has offered special difficulties. Since the intensity of the beam could not be kept quite constant, an indicator suitable for the entire dose range was desirable. In previous neutron experiments the doses have been measured by means of an Aerion condenser chamber (ZIMMER, 1938) or by a Victoreen r-dosimeter (AEBERSOLD and LAWRENCE, 1942). These arrangements were, however, not very convenient in connection with the high dosages necessary in the present experiments. Therefore, another method of dosimetry had to be used. The principle of this method is to place an Al plate together with the seeds inside the glass tube and to use the activity of the Al atoms produced by the neutron bombardment as a measure of the dosage applied (cf. AEBERSOLD and LAWRENCE, l. c., p. 35).

The primary reaction of the treatment is:

$$Al + n = Na^{24} + He$$

Na^{24} is an instable isotope with a half-life period of 14 hours, a rather convenient condition. The disintegration follows the scheme:

$$Na^{24} = Mg^{24} + e^{-} + \gamma$$

The α-, β- and γ-radiations thus produced, as well as any secondary radiation caused by the bombardment of the glass wall of the tube, are negligible from a biological point of view, compared with the high primary effect of the neutrons.

The α-particles are unable to penetrate the seed cover, and a certain amount of γ-radiation produced by the cyclotron itself passes through the Pb plates.

The intensity of the disintegration was measured 6—7 hours after the treatment and calculated back to the middle of the exposure time. In the investigation reported in this paper the activity was measured as the number of impulses given to a small β-tube, the cylindrically curved Al plates being placed upon it. The number of impulses obtained per gram Al and minute allowed of an accurate and reproducible comparison between different dosages. In this special case the Al plates were 2 mm thick with a weight of about 1,7 g.

The impulse figures determined in the above-mentioned way are dependent, unfortunately, on the individual β-tube used. For this reason another method of more general application was elaborated. The activity of the Al plates is here determined by an arrangement for β—γ-coincidence measurements. Thanks to the favourable disintegration scheme of Na^{24} the number of disintegrated atoms per gram Al and minute can be calculated. This number, given for the middle of the exposure time, is then used as a measure of the dose applied. This new unit, disintegration per gram Al and minute (abbreviated to *dis*), gives a good reproductivity for similar types of neutron treatments and does not depend on any special apparatus of measurement. The n-unit, used by American workers, has been estimated very approximately as equal to 130 *dis*. Since the absorption of neutrons varies in an entirely different manner from the absorption of X-rays, we have refrained from making any comparisons between the energy dissipation of neutrons and X-rays.

The dosimetry of the mutation experiments reported in this paper is based on the simple β-impulse method first applied. Though introducing a certain source of error, the dosages have been recalculated to *dis* units in order to make comparisons with future experiments possible. The five dosages were calculated at 40000, 80000, 120000, 150000 and 200000 *dis* units respectively.

X-rays. — The X-ray irradiation was performed in collaboration with Dr. A. FORSSBERG at the Radiophysics Institute, Stockholm. A complete description of the irradiation methods can be found in a paper published by FRÖIER in 1941. The dosages applied amounted to 5000, 10000, 15000, 20000, and 25000 r-units respectively, the intensity being 750 r/min.

The material of the present investigation consisted of seeds from the Svalöf two-rowed barley 01513 b_7. It is a homozygotized sister-line to Ymer barley [Maja $\times$ (Seger $\times$ Opal)], having a high productive capacity. Immediately before the treatments the seeds possessed a water content of 11,5 %. One single seed-size was used (sieve fraction: 2,5 to 2,75 mm).

The first field generation from X-rayed seeds is denoted as the X_1 generation. The chlorophyll mutations show up in the X_2 generation. Vital mutants of morphological type can be isolated in the same offspring, those of the physiological type are adequately analysed in the X_3. The corresponding expressions for neutrons experiments should be N_1, N_2 and N_3. The first, second and third generations after chemical treatments are denoted as M_1, M_2 and M_3, using the initial of mustard gas, the first chemical substance sufficiently analysed with regard to mutagenic activity. The initial C (»Chemical») is unsuitable, since it has already been applied in colchicine work.

RESULTS.

THE FIRST GENERATION.

Fig. 2 illustrates the conditions after an irradiation with X-rays. According to the curves shown, the number of harvested plants decreases rapidly down to 10 per cent with increasing dosage, whereas the average plant fertility falls slowly to a rather constant level of

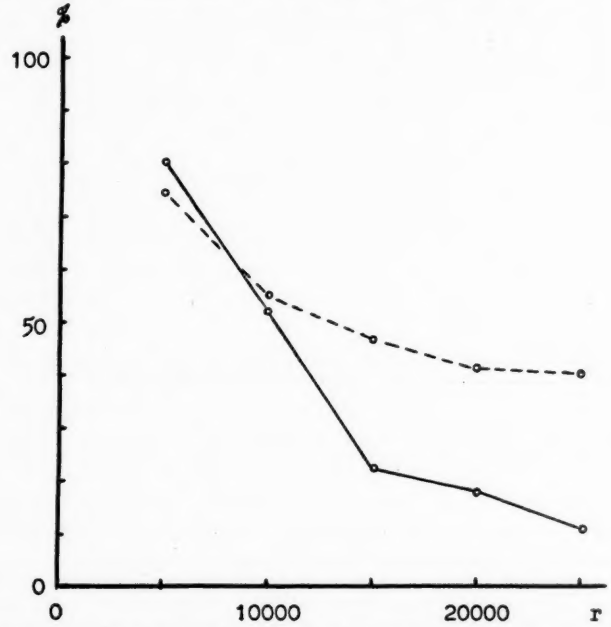

Fig. 2. The percentage of mature X_1 plants (unbroken line) and their average fertility (broken line) after different X-ray treatments.

40—50 per cent, below which level it is not apt to go. The figure depicts an X-ray effect of general significance.

In 1942, Table 1, GUSTAFSSON gave the corresponding data for Maja barley. He applied six dosages from 2500 to 25000 r. The percentage of harvested plants decreased to 8 % after 25000 r, but the average plant fertility with this dosage amounted to 67 per cent. For all dosages the fertility figures lay above the numbers of surviving plants. In 1943 FREISLEBEN and LEIN published some data concerning X_1 effects in the strain Haisa. In a first series they used six dosages ranging from 4000 to 14000 r. The number of harvested plants amounted to 12 per cent after 14000 r, the average plant fertility still being 50 per cent. The second experimental

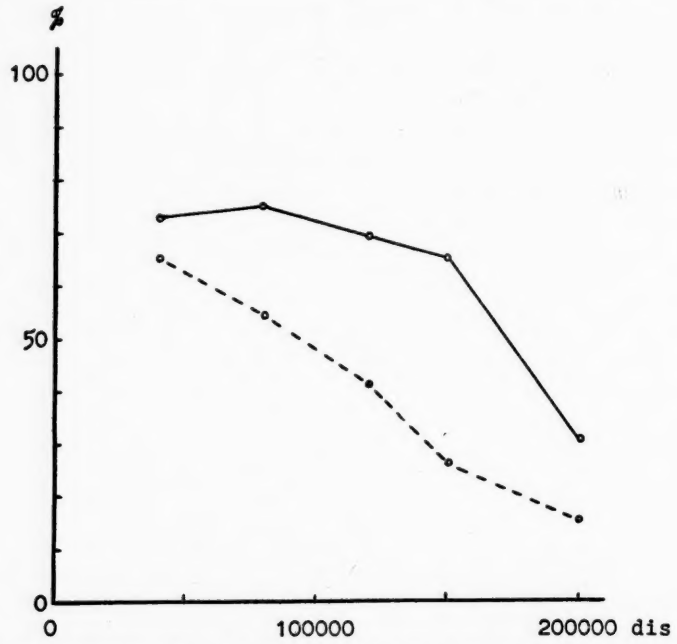

Fig. 3. The percentage of mature N_1 plants (unbroken line) and their average fertility (broken line) after different neutron treatments.

series covered four dosages from 10000 to 25000 r. After the highest dosage the number of harvested plants sank to 0,1 per cent but the average fertility was 36 per cent.

Some additional data are found in the papers of MÜNTZING (1940; strain Opal B) and GUSTAFSSON (1947; strain 01513 b_7). MÜNTZING used three dosages — 5000, 10000 and 15000 r. The harvested plants of the highest dosage amounted to 33 per cent, the average seed-setting being 73 per cent. GUSTAFSSON's data with regard to 01513 b_7 are not fully conclusive, since there was no figure relating to the plant number of the untreated material. Nevertheless, with every dosage the plant fertility lay well above the plant number. After 25000 r the average fertility was still 59 per cent, in spite of the fact that only one plant out of a total number of 374 had a normal seed-setting.

Thus, there is conclusive evidence proving the general validity of Fig. 2.

The neutron effects run differently (Fig. 3). Here the fertility curve entirely falls below the survival curve. The fertilities do not decline to the minimum value characteristic of X-rays but rapidly drop down from 65 per cent after 40000 *dis* to a value of 15 per cent after 200000 *dis*. The plant number curve, on the contrary, is fairly constant from 40000

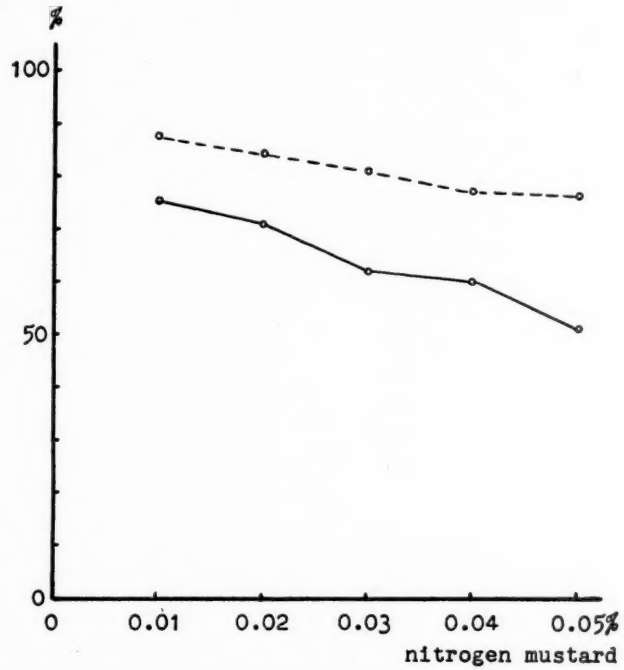

Fig. 4. The percentage of mature M_1 plants (unbroken line) and their average fertility (broken line) after treatments with nitrogen mustard.

to 150000 *dis*. With a dosage of 200000 *dis* the value still lies at 30 per cent. Irradiation experiments with X-rays have never produced a similar type of curve. No doubt, the curves depicted in Fig. 3 are representative of the neutron action, especially since another set of experiments gave exactly parallel data. It is a noticeable fact that neutrons easily produce chimaeras with white and yellow stripes even in the first generation.

Nitrogen mustard (Fig. 4) reacts differently from the neutrons. As discussed previously, laboratory experiments gave a low germination capacity after the treatment with 0,03 and 0,06 % solutions (Fig. 1), but the field series showed high germination rates, even with 0,04 and 0,05 % solutions. Consequently, the curves of Fig. 4 are incomplete. They indicate a slow and parallel decrease in the number of harvested plants and average plant fertility. Whether the curves are also different from those given by X-rays will be explored in future experiments.

TABLE 1. *Rates of mutation in the M_2, N_2 and X_2 generation.*

Treatment	No. of plant progenies	No. of spike progenies	No. of mutants	Mutation rate per spike progeny %
Control	100	183	—	0,0
0,01 % N-mustard	100	168	3	1,8
0,02 » »	100	203	—	0,0
0,03 » »	100	185	2	1,1
0,04 » »	100	205	2	1,0
0,05 » »	100	186	—	0,0
40000 dis	100	200	8	4,0
80000 »	100	189	10	5,3
120000 »	100	195	12	6,2
150000 »	91	166	11	6,6
200000 »	80	150	20	13,3
5000 r	100	227	10	4,4
10000 »	100	250	16	6,4
15000 »	64	214	4	1,9
20000 »	52	168	5	3,0
25000 »	30	130	6	4,6
Σ	1417	3019	109	—

THE SECOND GENERATION.

Mutation rates. — In the experiments reported here the control series produced no single case of chlorophyll mutation. In fact, the strain used was characterized by a conspicuous stability.

In essentials the behaviour of the five X-ray series is in accord with what is previously known from irradiation experiments in barley. The average mutation rates approximate a figure of $4 \cdot 10^{-6}$, calculated per r-unit and spike progeny. Similar rates were found by STADLER (1929; $4{,}9 \cdot 10^{-6}$ for Himalaya barley), GUSTAFSSON (1947, Table 4; $4{,}2 \cdot 10^{-6}$ for Golden barley) and D'AMATO and GUSTAFSSON (1948; $3{,}9 \cdot 10^{-6}$ for 01513 b_7). The figures are remarkably similar. The slightly smaller mutation rate in D'AMATO and GUSTAFSSON's experiments may depend on the very large seeds irradiated (the sieve fraction of 2,8 mm). We conclude that the mutation rate found by us entirely agrees with expectation.

The experiments do not show any distinct relationship between X-ray dosage and mutation rate (Table 1). Calculated per r-unit and spike progeny, the five mutation rates are 8,8, 6,4, 1,3, 1,5 and $1{,}8 \cdot 10^{-6}$, i. e. they do not lie at a constant level, in contrast to lethals and »visibles» obtained from irradiated sperm in *Drosophila.* Such a state of things is typical of seed material (FREISLEBEN and LEIN, 1943;

TABLE 2. *Types of mutation in the M_2, N_2 and X_2 generations.*

Treatment	No. of albina	No. of xantha	No. of alboviridis: Xantha-alba, albo-xantha	No. of alboviridis: Virido-albina albo-viridis	No. of alboviridis: Virido-xantha, xanth-viridis	No. of viridis	No. of tigrina	No. of striata	No. of maculata	No. of others
0,01 % N-mustard	—	—	—	—	—	3	—	—	—	—
0,02 » »	—	—	—	—	—	—	—	—	—	—
0,03 » »	—	—	—	—	—	2	—	—	—	—
0,04 » »	1	—	—	—	—	1	—	—	—	—
0,05 » »	—	—	—	—	—	—	—	—	—	—
40000 dis	5	—	—	1	—	2	—	—	—	—
80000 »	6	1	—	—	—	3	—	—	—	—
120000 »	5	1	—	—	—	5	—	—	—	1
150000 »	5	1	—	—	1	2	—	—	—	2
200000 »	12	1	—	2	1	2	—	1	1	—
5000 r	4	1	—	—	2	2	1	—	—	—
10000 »	10	2	—	1	1	2	—	—	—	—
15000 »	2	—	—	—	1	1	—	—	—	—
20000 »	1	—	1	—	—	3	—	—	—	—
25000 »	3	1	—	1	—	1	—	—	—	—
Σ	54	8	1	5	6	29	1	1	1	3

GUSTAFSSON, 1947, pp. 42—46). The first-mentioned two authors found that the absolute mutation frequencies increased up to a dose of 8000—10000 r. With still higher dosages the absolute frequencies decreased again. Calculated per r-unit, they declined continuously. Working with albina mutations in barley, GUSTAFSSON similarly found a decrease in mutation rate (calculated per r-unit) for dosages from 2500 to 15000 r. With still higher dosages of 20000 and 25000 r the mutation rates lay approximately constant (l. c., Fig. 10). This is exactly what happens in our present material with regard to the sum of all chlorophyll mutations. There is a steep fall in the mutation rate from 5000 to 15000 r calculated per r-unit. Thereafter there is a slight increase, statistically insignificant.

Like the authors referred to above, we regard selective conditions during ontogenesis as responsible for this absence of proportionality. The seed is a multicellular structure. The sperm is not. Hence, the two kinds of material ought to react differently to the irradiation. This problem will be further discussed below.

Unfortunately the applied water solution of nitrogen mustard covered a rather small range of concentrations (Fig. 4). Seven mutants were recorded in the progeny of 500 M_1 plants, or 947 M_1 spikes. This makes a total mutation frequency of 1,4 % per plant progeny, or 0,74 % per spike progeny, i. e. figures distinctly higher than in any spontaneous material previously analysed. In fact, there is a significant difference between mustard gas-treated and control material.

GUSTAFSSON (1947, p. 42) found that an appropriate heat treatment or ageing of the seed increased the mutation frequency by ten or eleven times compared with the spontaneous mutation rate. This increase, caused by environmental changes, corresponds to an X-ray dosage of 500 r. Comparing the effects of X-rays and nitrogen mustard, we find that the application of this chemical causes an average mutation rate of 0,48 per cent if calculated per spike progeny and for treatment with an 0,01 % solution lasting forty minutes. Consequently, this standard treatment answers to an X-ray effect of approximately 1200 r-units. This indicates that the chemical induction of mutations by means of nitrogen mustard is distinctly superior to the well-known effects of heat treatment and ageing. In fact, it is comparable to X-ray irradiation for plants as well as for animals (cf. AUERBACH and ROBSON, 1947).

Neutrons deviate considerably from X-rays with regard to the interdependance of mutation rate and dosage. Calculated per *dis* and spike progeny, the five mutation rates are as 1,0, 0,7, 0,5, 0,4, and $0{,}7 \cdot 10^{-6}$,

i. e. figures in reasonable agreement with the linear relationship shown to occur in *Drosophila* by ZIMMER and TIMOFÉEFF-RESSOVSKY, 1938.

The average mutation rate after neutron treatment amounts to $0{,}66 \cdot 10^{-6}$ per spike progeny and *dis*. In consequence, circa six disintegrations per gram Al and minute correspond to one r-unit as to the mutation effect in cereal seed.

Mutation types. — The normally occurring chlorophyll mutants can be divided into five phenotypical groups: albina, xantha, alboviridis, viridis, and tigrina. In addition, there are the rare striata and maculata groups as well as some rare mutants of deviating type. Albina and viridis mutants are in the majority. The X-ray experiments of GUSTAFSSON (1940, 1947), FREISLEBEN and LEIN (1943), as well as D'AMATO and GUSTAFSSON (1948) indicate the following average percentages: albina — 51 %, xantha — 6 %, alboviridis — 12 %, viridis — 28 %, tigrina — 1—2 %, others — less than 1 %. Type of material, water content, pre-treatments, etc. change the percentages more or less.

The series analysed here give the following proportions: albina — 49 %, xantha — 10 %, alboviridis — 17 %, viridis — 22 %, tigrina — 2 %, others — 0 %, i. e. a rather fair agreement with the above-mentioned data gathered from various sources.

In their cited paper of 1948 D'AMATO and GUSTAFSSON concluded that a pre-treatment with weak colchicine solutions was able to widen the range of mutability, very rare or previously unknown mutants being more or less frequent. The untreated seed series, irradiated with 7500 r, produced eighteen mutants: 8 albinas, 1 xantha, 1 alboviridis, and 8 viridis types. The contrast between colchicine and dry series gave P figures less than 0,01. Our new material strengthens the conclusions drawn:

	Common mutants	Rare mutants	Very rare mutants
X_2 — 1946—1947	89 %	11 %	*0 %*
X_2 — 1947—1948	71	27	*2*
X_2 — colchicine series 0,1 + 0,05 % sol.	66	23	*12*
X_2 — colchicine series 0,01 + 0,005 % sol.	53	21	*26*

The X-ray material published here does not indicate any change of mutation type with increasing dosage. The proportions of different mutants are approximately identical after high, as well as after low dosages.

The mutation proportion in the case of neutrons is as follows: albina — 54 %, xantha — 7 %, alboviridis — 8 %, viridis — 23 %, very rare mutants — 8,2 %. The figures of Table 2 go to show that such

mutant types as striata, maculata and others are more easily produced by neutrons than by X-rays, but the data do not allow of definite conclusions. Nevertheless, it is rather striking that neutrons *did* produce two independent cases of a mutant previously not observed, combining characters of tigrina and maculata. Furthermore, there is some indication that the rare types preferably arise after high neutron dosages, since there is no single case with 40000 and 80000 *dis*, one with 120000 *dis*, and two with each dosage of 150000 and 200000 *dis*.

Much more interesting, for the present, is the different behaviour of the mustard gas series. Here seven mutants appeared, six of which belong to the viridis group. Only one is an albina. In spite of the low mutant number, there is a significant difference between the proportion of albina to viridis after treatment with nitrogen mustard (1 : 6), after X-ray irradiation (20 : 9), and after neutron treatment (33 : 14). P is equal to 0,013 and 0,008 respectively. (These P values are based on the exact methods worked out for 2 × 2 tables; BONNIER and TEDIN, 1940, pp. 226—227; KENDALL, 1945, p. 304). Consequently we may infer that nitrogen mustard, relative to the effects of X-rays and neutrons, enhances the production of viridis types but decreases that of albina.

CONCLUSIONS AND DISCUSSION.

By this investigation it has been shown that nitrogen mustard can also be used in agricultural plant species for the induction of mutations. A 0,01 % solution, applied to dormant seeds for 40 minutes, corresponds to a treatment with 1200 r-units and is more effective than extreme heat and ageing. What is even more interesting, however, is that nitrogen mustard mostly produces chlorophyll mutations of the viridis type, very rarely of the albina type. The statistical analysis of the data suggests a non-random production of mutations by this agent. Quite recently more data supporting this conclusion have emerged.

That the mutation process may run somewhat differently for mustard gas and X-rays, is nothing peculiar, since it has been proved that translocations are rare with mustard gas, whereas lethals and deficiencies are common (cf. AUERBACH, ROBSON and CARR, 1947). The same treatment as produces 9 per cent sex-linked recessive lethals in *Drosophila* gives no more than 0,5 per cent interchanges of the second and third chromosomes, against a value of 6 per cent calculated in the case of X-rays. Moreover, mustard gas substances are apt to produce gene instabilities that give rise to mosaic formation in later generations.

In their recent paper of 1947 (p. 213) DARLINGTON and KOLLER emphasize the cytological similarity of mustard gas and X-ray effects and use it as an objection to the target theory. We consider, on the contrary, that the mutagenic actions of these agents are distinctly different.

The same appears to be valid with regard to the mutual behaviour of neutrons and X-rays. Physically these types of radiation are entirely distinct. Neutrons act by the formation of a comparatively small number of protons, X-rays by the ejection of numerous electrons. The ion density is considerably higher in a proton than in an electron track. Sex-linked recessive lethals in *Drosophila*, usually regarded as one-hit events, are produced less frequently by neutrons than by X-rays (calculated for »similar» doses; ZIMMER and TIMOFÉEFF-RESSOVSKY, 1938). The reason is, the authors argue, that with neutrons, as well as with α-rays, though usually not with X-rays, more than one ionization occurs in the same target area. If, on the other hand, a biological reaction requires two or more ionizations, densely ionizing radiations will be more effective than X- and γ-rays (l. c.; also LEA, 1946).

Several authors have concluded that chromatid and chromosome breaks leading to chromosome aberrations are more liable to occur after neutron than after X-ray treatments (LEA, l. c., Table 2, p. 238). Furthermore, the yield of chromosome exchanges is said to be proportional to the first power of the dose if neutrons are considered, but proportional to the 3/2 power with X-rays. From these and other data LEA advanced the idea that chromosome breaks are caused by several ionizations. Protons and α-rays cause a break with a probability almost equal to unity. In the case of X-rays the minimum number of ionizations that with a probability of unity will cause chromosome breakage is 15—20. In accordance with LEA's conclusions neutrons induce more chromosome structural changes than X-rays.

The present investigation confirms the view that neutrons imply an active mutagenic agent in agricultural plants also. It is evident that neutrons, better than X-rays, fulfil the requirements for a linear relationship of dose and mutation rate. Unfortunately, there is no possibility at present of converting the neutron dosages into r-units. The high X-ray dosage of 25000 r, which but few plants survive, induces 20 per cent mutations calculated per plant progeny, and 4,6 per cent if calculated per spike progeny. The highest neutron dosage (200000 *dis*) shows three times as many first generation plants but, in addition, 25 and 13,3 per cent mutations, calculated per plant or spike progeny

respectively. Thus, high dosages of neutrons are at least as effective as X-rays. Comparing the mutagenic effects of the radiations, we conclude that 6 *dis* are approximately comparable to 1 r.

There seems to be no doubt that X-rays and neutrons act quite differently in barley. Neutrons cause, in the first place, the formation of chimaeras in the first generation, many plants carrying longitudinally white or yellow stripes, a phenomenon very infrequent in X-ray experiments. Equally striking is the inverse relation of surviving plants and average plant fertilities. With X-rays the number of harvested plants rapidly decreases to one or a few per cent, although the average fertility does not easily fall below 40—50 %. In neutron experiments a great number of plants having a low fertility are able to survive. The physical reason is probably that the barley seeds are traversed, relatively seen, by many more ionizing particles in X-ray than in neutron experiments. More cells are affected by X-rays. Owing to the densely ionizing capacity of protons, however, the individual cells will become more damaged by neutrons than by X-rays. In X-ray irradiation the damages are spread over the whole tissue but are not so severe in the individual cells. The consequence is just what happens: with high neutron dosages more seedlings survive but simultaneously the chromosome disturbances leading to decreased fertility are more potent.

This is, we consider, a result which will turn out rather valuable in practical work. Mutations connected with gross chromosome aberrations and re-arrangements, i. e. those caused by several ionizations, will be more easily produced by neutron than by X-ray treatment. Thus, drastic mutants which change the external appearance considerably and according to several findings are associated with high first generation sterility may be expected to be frequent in neutron series. There is already some indication, although so far not statistically significant, that neutrons induce rare mutation types more frequently than do X-rays. However, these are problems that will be attacked in future experiments.

SUMMARY.

Dormant seeds of barley were treated with solutions of nitrogen mustard and the effects of this substance were examined in the first (M_1) and the second (M_2) generation. The mutation rate after a treatment with 0,01 % solution for 40 minutes corresponds approximately to an effect of 1200 r-units. Nitrogen mustard induces a higher mut-

ability than heat treatment or ageing. The mutants appearing are predominantly of the viridis type, albinas being rare.

Neutrons obtained from a cyclotron were applied to dormant seeds. The dosages were measured by a method involving the change of aluminium into a radioactive Na isotope having a half-life period of 14 hours. The β- and γ-radiations were determined, permitting the dosage to be measured in disintegrations per gr Al and minute. Neutrons distinctly differ from X-rays with regard to their immediate effects. Chlorophyll chimaeras already appear in the N_1 but usually not in the X_1 generation. High X-ray dosages decrease the number of mature plants to one or a few per cent, but the average fertility remains at a level of 40—50 %. Neutrons give numerous surviving plants after high dosages as well but, on the other hand, greatly decrease the fertility. These results are, the authors consider, dependent on the difference in ion density. In mutagenic action 6 disintegrations answer to 1 r-unit. Some data indicate a specially wide mutation range in the case of neutrons.

The conclusions are drawn that both mustard gas substances and neutrons may be used with advantage in mutation work dealing with agricultural plants. To a certain extent these agents differ from X-rays in their genetical action.

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SOME INTERSPECIFIC CROSSES IN CALAMAGROSTIS AND THEIR EVOLUTIONARY CONSEQUENCES

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IN 1941 a hybrid was obtained in a cross between the two amphimictic species *Calamagrostis arundinacea* (L.) ROTH (2n = 28) and *C. epigeios* (L.) ROTH (2n = 56). The former species was used as mother. The chromosome number of the hybrid was determined as 2n = 42. The F_1 plant did not flower until 1944, but since that year it has developed about 200 panicles or more every summer. The hybrid looks rather like *C. epigeios*, but its laminae have the same pale green shade as is typical of *C. arundinacea*.

When in 1945 the meiosis of the hybrid was examined, great similarities to the apomictic *Calamagrostis* species were found (NYGREN, 1946, p. 239). On the male side there were serious disturbances, most P.M.C.'s forming plasmodia by fusion of solitary P.M.C.'s or whole loculi. In such cases, as a rule, no divisions at all started, the plasmodia degenerating sooner or later. The few P.M.C.'s going trough meiosis developed pollen containing most chromosome numbers between 21 (or lower numbers) and 84. On the female side the meiosis was as disturbed as on the male side, but here, besides the reductional division, the *mitotic* one occurred, which is characteristic of many apomictic genera (cf. GUSTAFSSON, 1946).

Some panicles of the hybrid were bagged, but no seedlings were obtained. On the other hand, 49 F_2 plants were obtained, with some difficulties, after free flowering. In 1947 46 of these plants survived; 43 of them flowered this summer. It could be established that though most of the F_2 plants looked like *C. epigeios* or the original F_1 hybrid — *C. arundinacea* × *C. epigeios* — eight plants showed remarkable similarities to *Calamagrostis varia* (SCHRAD.) HOST., a Middle-European—Asiatic species, which in Scandinavia only occurs in the island of Gotland. The chromosome numbers of these eight plants deviated, however, from that of *C. varia*. The F_2 plants had 2n = 35, 37, or 38, while a true *C. varia* from nature has 2n = 28.

I. MORPHOLOGICAL FEATURES OF CALAMAGROSTIS ARUNDINACEA, C. EPIGEIOS, THEIR HYBRID AND THE F_2 PLANTS.

1. PARENTS.

The mother plant, *C. arundinacea*, comes from Röstånga in the province of Scania in the South of Sweden. The species is tufted and characterized by long, arcuated leaves, short ligula and hair pads where the laminae pass into the sheath. The lemmas are almost as long as the glumes, they have long genuflexed awns fixed to the base, and are about three times as long as the callus hairs.

The father plant in the actual cross, *C. epigeios*, comes from Härnösand in the Middle of Sweden. This species develops stolons in great numbers, and has a long ligula, but no hair pads between the lamina and the sheath. The glumes are thin, drawn out into a subulate point, and about three times as long as the lemmas, which have short, straight awns fixed in the middle part. The callus hairs are longer than the lemmas.

2. THE F_1 PLANT.

The hybrid is tufted as *C. arundinacea*, but develops stolons as *C. epigeios*. The characteristic features of the plant are: ligula long, hairiness between the lamina and the sheath slight, length of the lemmas intermediate between the parents, callus hairs of the same length as the lemmas, and awns long, genuflexed and fixed to the base. The plant is very vigorous, as is evident from the great number of panicles developed every summer (more than 200). The colour of the plant is at first pale green as in *C. arundinacea*, later on it becomes darker and more reminiscent of *C. epigeios*. The laminae are arcuated as in *C. arundinacea*, some of them have solitary hairs just as this species. The features of the two species and their hybrid have been brought together in Table 1.

Though only 14 of the 42 chromosomes in the hybrid come from *C. arundinacea*, the latter species dominates the characters of the F_1 plant. The tufted manner of growing, the long, arcuated leaves, the hairiness between the lamina and the sheath, the length, form and fixing point of the awns are all features characteristic of *C. arundinacea*. The only character of *C. epigeios* which agrees with the hybrid is the length of the ligula. The length of lemmas and the relation between the lemma and the callus hairs are intermediate between the parental species.

TABLE 1. *Characteristic features of Calamagrostis arundinacea, C. epigeios (2n = 56) and their artificial hybrid.*

Species	Number of nodes	Length of ligula	Hairiness Lamina/sheath	Length of		Relation of lemma/callus h.	Characters of the awns		
				Glumes	Lemmas		Length	Form	Fixing point
C. a.	3,0	3,8 mm	Strong	5,3 mm	4,5 mm	4 : 1	8,3 mm	Bent	At the base
C. ep.	3,0	9,6	0	6,5	2,5	1 : 1,5	1,5	Straight	In the middle part
Hybrid	3,3	7,1	Slight	5,4	3,4	1 : 1	5,1	Bent	At the base

All values are the averages of 20 measurements on the plants. The length of the ligula has been measured on the uppermost laminae of the straw, the length of glumes, lemmas, and awns of the same panicle. — *C. a.* = *Calamagrostis arundinacea*, *C. ep.* = *Calamagrostis epigeios.*

3. THE F_2 PLANTS.

The mother in the original cross, *Calamagrostis arundinacea*, is extremely allogamous, while the father, *C. epigeios* with 2n = 56, often behaves as an autogamous species in contrast to its chromosomal race with 2n = 28, which is allogamous (NYGREN, 1946, p. 223). In F_2 there are scarcely two plants that look like each other.

Only 23 out of the 43 plants could be completely investigated. Their features have been brought together in Table 3. A survey of the 43 plants is to be found in Table 2.

There are no fewer than 16 out of 19 plants resembling *C. epigeios* (= 84,2 %) among the descendants with chromosome numbers above 42, whereas 2 resemble *C. arundinacea* × *C. epigeios* and only one *C. arundinacea*. The plants with lower chromosome numbers than 42 »segregate» into four groups: those resembling *C. epigeios*, 9 out of 22 (= 40,9 %); *C. varia* 7 (= 31,8 %); *C. arundinacea* × *C. epigeios* 4 (= 18,2 %); *C. epigeios* × *C. varia* 2 (= 9,1 %). In the cases where an egg-cell with an unreduced chromosome number has been fertilized, the descendant resembles *C. epigeios*. The reason for this may be that some *C. epigeios* plants having the chromosome number 2n = 56 grew close to the F_1 plant. The chances for these plants to pollinate the F_1 plant were not so great, however, as the latter did not flower until one or two weeks later than the actual *C. epigeios* plants. *C. arundinacea* and *C. varia* flowered about one month earlier and, as *C. epigeios* too,

TABLE 2. *Survey of F_2 plants of the cross Calamagrostis arundinacea × C. epigeios.*

Plant No.	2n	Habit	Manner of growth		Relation : glume/awn	Hair pads between the lamina and the sheath
			Tufted	Stolons		
201— 1	56	*C. ep.*	0	+	Awns somewhat longer	0
2	73	*C. a.* × *ep.*	+	0	» » »	0
4	65	*C. ep.*	0	+	Glumes longer	0
5	51	*C. ep.*	0	+	» »	0
6	72	*C. ep.*	0	+	» »	0
7	35	*C. a.* × *ep.*	+	0	Awns longer	+
8	71	*C. ep.*	0	+	Glumes longer	0
9	38	*C. v.*	+	0	Awns as long as the glumes	+
10	46	*C. ep.*	0	+	Glumes longer	0
11	51	*C. ep.*	0	+	» »	0
12	71	*C. ep.*	0	+	» »	0
13	37	*C. v.*	+	0	Awns longer	0
14	71	*C. ep.*	+	+	Glumes longer	0
15	36	*C. ep.*	+	+	» »	0
16	37	*C. ep.*	0	+	» »	0
17	37	*C. ep.*	+	+	Awns longer	0
18	37	*C. v.*	+	0	» »	+
19	35	*C. ep.* × *v.*	+	+	Awns as long as the glumes	+
20	35	*C. ep.*	0	+	Glumes longer	0
21	39	*C. ep.*	+	+	Awns somewhat longer	0
22	37	*C. v.*	+	0	Awns much longer	+
23	72	*C. ep.*	0	+	Awns longer	0
24	71	*C. ep.*	0	+	Awns as long as the glumes	0
25	78	*C. ep.*	0	+	Glumes longer	0
26	37	*C. ep.* × *v.*	+	0	Awns somewhat longer	+
27	60	*C. a* × *ep.*	+	0	Awns much longer	0
28	36	*C. ep.*	0	+	Awns longer	0
29	36	*C. ep.*	0	+	Glumes longer	0
30	38	*C. v.*	+	0	Awns much longer	+
31	36	*C. ep.*	0	+	Glumes longer	0
33	35	*C. a.* × *ep.*	+	+	» »	+
34	58	*C. ep.*	0	+	» »	0
35	58	*C. ep.*	0	+	» »	0
36	36	*C. ep.*	0	+	» »	0
37	35	*C. v.*	+	0	Awns much longer	0
38	62	*C. ep.*	0	+	Awns somewhat longer	+
39	58	*C. a.*	+	0	Very long awns	+
40	71	*C. ep.*	0	+	Glumes longer	0
42	38	*C. v.*	+	0	» »	0
43	40	*C. a.* × *ep.*	+	+	Awns longer	0
44	—	*C. ep.*	0	+	Glumes longer	0
45	—	*C. v.*	+	0	Awns as long as the glumes	+
46	37	*C. a.* × *ep.*	+	0	Awns longer	0

they are never remontant. On these accounts it is probable that only pollen from the F_1 plant itself has fertilized the egg-cells. In all species investigated of *Calamagrostis* pollen grains with the chromosome numbers 14, 21, or 28, etc. seem to be the only functional ones. It is probable that pollen grains are formed in the F_1 plant containing whole chromosome sets from one or both of the parents, thus holding 14 *arundinacea* chromosomes, 14 or 28 *epigeios* chromosomes, 14 *arundinacea* and 14 *epigeios* chromosomes, and so on. As the percentage of morphologically good pollen in the F_1 plant is lower than 0,5 %, it is a very plausible possibility that only the chromosome combinations just mentioned give pollen that is utilizable. The deviating chromosome numbers in the offspring depend on irregularities on the female side. It is possible that most unreduced egg-cells have been fertilized by pollen grains holding about 28 *epigeios* chromosomes and that they have thus given rise to embryos having about 70 chromosomes. Reduced egg-cells have in most cases been fertilized by pollen having 14 chromosomes, probably 14 *arundinacea* or 14 *epigeios* chromosomes. Most such egg-cells contain chromosome numbers between 21 and 28. If 14 is added to these numbers, we obtain $21 + 14 = 35$ (5 plants), $22 + 14 = 36$ (5 plants), 37 (7 plants), 38 (3 plants), 39 (1 plant), and 40 (1 plant). The two combinations remaining have not been realized.

The segregation of the F_2 could of course not be estimated on account of the small number of plants. Some details may be worth pointing out, however. We can state that the tufted F_1 plant has given rise to 21 tufted and 22 non-tufted F_2 plants. In 12 of the 43 plants tested there appear hairs between the lamina and the sheath, while 21 plants have awns longer than the lemmas. Most species of the genus *Calamagrostis* have stolons, e. g., *C. epigeios* to a great extent and *C. arundinacea* to a small extent. In 29 F_2 plants the stolons were very well developed. In all the 23 carefully investigated F_2 plants the length of the awns is greater than in the paternal species, *C. epigeios*, and in no fewer than 18 plants the fixing point of the awn is near the base of the lemma, resembling the situation in *C. arundinacea*, while *C. epigeios* has it fixed in the middle part.

The F_2 plants similar to *C. varia* are of interest from an evolutionary point of view. In order to make a comparison possible between the

C. a. = *Calamagrostis arundinacea*, *C. ep.* = *Calamagrostis epigeios*, *C. v.* = *Calamagrostis varia*. Plants Nos. 9, 12, 13, 18, 22, and 37 are all darker than both parents, Nos. 1, 2, 6, 11, 14, 30, and 45, extremely pale. The awns have been considered to be longer than the glumes if they protrude.

TABLE 3. *Features of F_2 plants of Calamagrostis arundinacea × C. epigeios.*

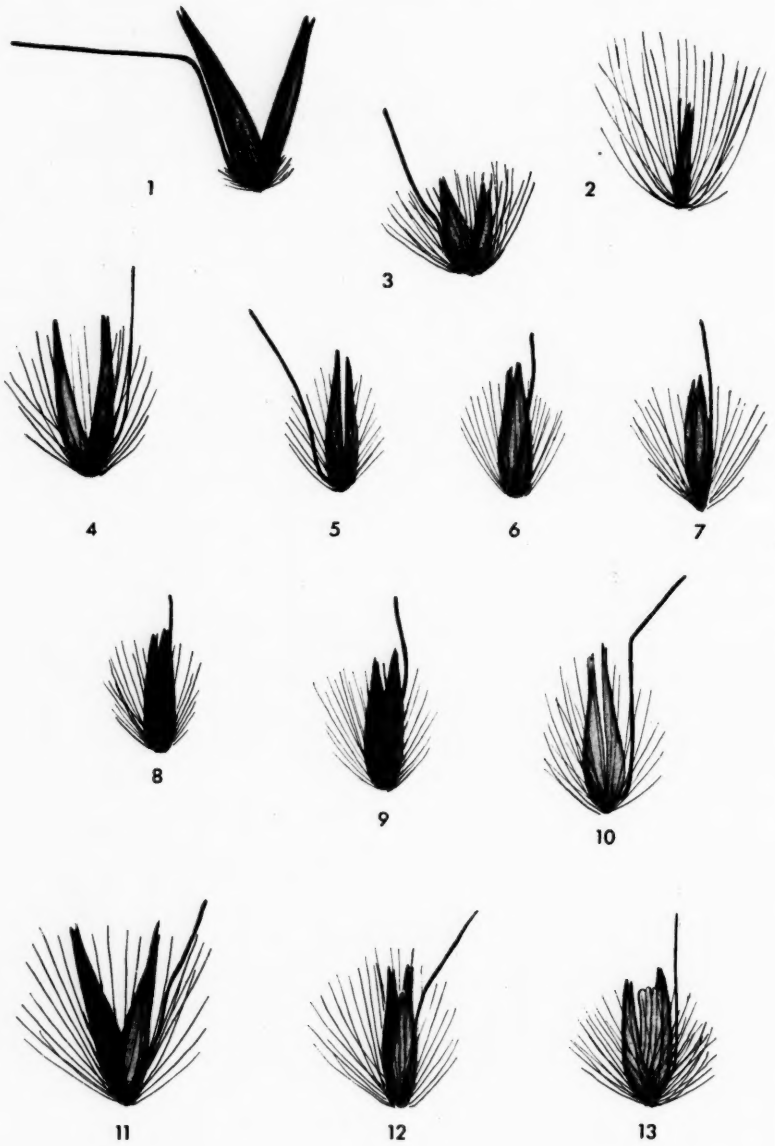
Plant No.	2n	Length of ligula	Length of glumes	Relation: lemma/callus hairs	Characters of the awns: Length	Fixing point	Form
201— 4	65	6,5 mm	6,0 mm	Callus h. longer	4,8 mm	Near the base	Bent ±
5	51	7,0	4,8	» » »	2,3	In the middle	Straight ±
6	72	7,0	6,0	» » »	4,0	Near the base	» »
8	71	7,2	5,4	» » »	3,9	» » »	»
9	38	4,0	5,0	Lemma somewhat longer	4,0	» » »	Bent ±
10	46	5,5	5,2	Callus h. longer	2,8	In the middle	Straight ±
11	51	4,0	4,3	» » »	2,6	» » »	» »
12	71	6,5	6,6	» » »	4,6	Near the base	» »
13	37	4,1	5,0	Lemma somewhat longer	4,3	» » »	Bent ±
15	36	4,6	5,5	Callus h. longer	3,7	In the middle	» »
18	37	5,2	4,9	Lemma longer	4,2	Near the base	Straight ±
19	35	3,0	4,6	» »	4,1	» » »	» »
21	39	4,2	4,6	» »	3,9	» » »	» »
22	37	5,0	5,0	» »	4,8	» » »	» »
25	78	6,0	6,5	Callus h. longer	3,2	In the middle	» »
26	37	4,2	4,7	Lemma longer	4,0	Near the base	»
27	60	5,5	4,6	» »	3,2	» » »	»
28	36	3,0	4,9	» »	3,2	» » »	» »
31	36	5,0	4,7	Callus h. longer	3,1	» » »	» »
37	35	3,0	5,5	Lemma longer	4,4	» » »	» »
39	58	4,1	5,5	» »	5,4	» » »	» »
42	38	4,9	5,7	» »	3,8	» » »	» »
45	—	4,0	5,0	» »	4,3	» » »	Bent ±

The fixing point of the awns varies somewhat in different lemmas of the same panicle. The diagnoses mean that more than 50 % of the lemmas of a panicle behave in that manner. The form of the awns varies a great deal. The sign + means that the awn is bent, —, that it is straight. If bent as well as straight awns are present, both signs have been used. The diagnoses »straight» or »bent» mean that more than 50 % of the awns measured belong to this group. All values are based on 20 measurements.

TABLE 4. *Comparison between features of Calamagrostis varia* (SCHRAD.) HOST. *and some F_2 plants of the cross C. arundinacea × C. epigeios.*

Species	Locality	Length of ligula	Length of glumes	Relation : lemma/callus hairs	Length of awns	Fixing point of awns	Form of awns	2n
C. v.	Gotland	4,0 mm	5,1 mm	Lemma somewhat longer	4,5 mm	Near the base	Bent or straight	28
C. v.	Innsbruck	4,0	4,9	» as long as the c. h:s	4,0	» » »	Straight	28
C. v.	Zürich	4,0	4,8	» somewhat longer	4,0	» » »	Str. or b. in rel. 3 : 2	28
C. a. × *ep.*	201 — 9	4,0	5,0	» » »	4,0	» » »	Bent or straight	38
C. a. × *ep.*	201—13	4,1	5,0	» » »	4,3	» » »	Bent or straight	37
C. a. × *ep.*	201—18	5,2	4,9	» » »	4,2	At the base	Straight	37
C. a. × *ep.*	201—22	5,0	5,0	» » »	4,8	» » »	Straight or bent	37
C. a. × *ep.*	201—37	3,0	5,5	» » »	4,4	» » »	Straight	35
C. a. × *ep.*	201—42	4,9	5,7	» » »	3,8	» » »	Straight or bent	38
C. a. × *ep.*	201—45	4,0	5,0	» » »	4,3	» » »	Straight or bent	—

C. v. = *Calamagrostis varia*, *C. a.* = *Calamagrostis arundinacea*, *C. ep.* = *C. epigeios*. The diagnoses of the fixing point of the awn are often a matter of taste, as different lemmas in the same panicle differ rather much in this respect. All values are based on 20 measurements.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features of these plants and those of *Calamagrostis varia* from nature, values have been worked out for the latter species from Gotland (Sweden), Innsbruck, and Zürich (Table 4), and parallelled with the values of the plants of interest in Table 3.

In »Illustrierte Flora von Mittel-Europa» HEGI (1908) separates *C. arundinacea* and *C. varia* on the length of the callus hairs (p. 230). In *C. varia* they are said to be richly developed and generally as long as the lemmas, while *C. arundinacea* is claimed to have scantily developed hairs, only $^1/_4$ as long as the lemmas. ASCHERSON and GRAEBNER (1898—1902) give the following description of the most characteristic features of *C. varia* (p. 209): »Die Hüllspelzen etwa um 1 mm überragende, schwach oder deutlicher gekniete Granne etwa auf $^1/_4$ der Länge der Deckspelze abgehend. Haare meist so lang als die Deckspelze, seltener erheblich kürzer». The form of the awn varies, however, as can be seen from Table 4. By comparing Figs. 11—13 with Figs. 4—10 it is easy to establish the facts that the form and length of the lemmas, the form and fixing point of the awns, and the length of the callus hairs in *C. varia* lie within the limits of variation in the F_2 plants, and that they show remarkable similarities to corresponding parts in the latter. In addition to this, it may be noted that the manner of growth and the colour of the plants are exactly the same as in *C. varia* from nature.

In the work of HERIBERT NILSSON (1918) on *Salix*, the term »extravagante Typen» was formulated to characterize »dergleichen Nachkommen einer Kreuzung, an denen man nicht mehr die Eltern erkennen kann» (p. 57). On p. 37 of his work we find an example of this in the appearance of two individuals resembling *Salix repens* in the F_2 of the cross *S. viminalis* × *caprea*: »Die beiden Individuen dieses Typus sind so ausgeprägtem *repens*-Wuchs, dass keiner, der sie wildwachsend gefunden hätte, bezweifeln würde, dass *S. repens* eine der Komponenten dieser Bastardindividuen sei». On p. 61 we read: »Aus der Kreuzung *viminalis* × *caprea* gehen also Individuen hervor, die besser dem gedachten intermediären Typus *aurita* × *viminalis* entsprechen als der artifizielle Bastard zwischen diesen Arten». Results similar to those

Figs. 1—13. »Flowers» (lemma, palea, and callus hairs) of *Calamagrostis arundinacea* (L.) ROTH, *C. epigeios* (L.) ROTH, *C. arundinacea* × *epigeios*, F_1 and 7 F_2 plants, and *C. varia* (SCHRAD.) HOST. — 1, *C. arundinacea*, 2n = 28. — 2, *C. epigeios*, 2n = = 56. — 3, *C. arundinacea* × *C. epigeios*, 2n = 42. — 4, F_2, 201—9, 2n = 38. — 5, F_2, 201—13, 2n = 37. — 6, F_2, 201—18, 2n = 37. — 7, F_2, 201—22, 2n = 37. — 8, F_2, 201—37, 2n = 35. — 9, F_2, 201—42, 2n = 38. — 10, F_2, 201—45, 2n = ?. — 11, *C. varia*, 2n = 28, Visby, Gotland, Sweden. — 12, *C. varia*, 2n = 28, Innsbruck, Austria. — 13, *C. varia*, 2n = 28, Zürich, Switzerland. — Magnif. 5,5 ×.

obtained in *Salix* and *Calamagrostis* can probably be found by careful investigation in most critical genera. According to the opinion of the present writer, the phenomenon does not at all depend on mere chance. It will be discussed later on.

II. CYTOLOGICAL STUDIES IN CALAMAGROSTIS ARUNDINACEA, C. EPIGEIOS, THEIR HYBRID AND THE F_2 PLANTS.

1. PARENTS.

Meiosis in the mother plant, *C. arundinacea*, is quite regular on the male as well as on the female side, 14 bivalents appearing at metaphase I. In the father species, *C. epigeios*, there are slight disturbances in form of quadrivalents on the male side. In 13 P.M.C.'s 3 quadrivalents and, for the rest, only bivalents were counted (NYGREN, 1946, p. 144). The female side could not be analysed, but, according to crossing experiments, it seems to behave like the male side.

2. THE HYBRID.

In the hexaploid hybrid meiosis was much disturbed on the male side. A survey of mine in 1946 (p. 239) gave the following types of division:

I. *Divisions occur.*

A. P.M.C.'s mutually free.

1. Normal P.M.C.'s	(a) Normal meiosis with up to 21 bivalents. Very variable in number of univalents. Passes over into type (c).
	(b) Quadrivalents occur in small number, 1—3.
	(c) Asynaptic P.M.C.'s with 42 univalents. Forms transitions to (a).
2. Synpolyploidy.	P.M.C.'s occur with the chromosome number 84 or higher numbers. Bivalents are only rarely formed.
3. Pseudohomeotypic division.	This division type (GUSTAFSSON, 1935) occurs in a remarkably large number of P.M.C.'s. Often the chromosomes have divided at different times, and therefore all do not lie simultaneously in the equatorial plane.
4. Semiheterotypic division.	This division type is very rare, but occurs in certain P.M.C.'s.

B. Plasmodia.

1. Fusion plasmodia.	Such plasmodia are common. The whole loculus may be included through disintegration of all walls between the P.M.C.'s. If divisions start, they usually only go on to the diplotene-diakinesis stage. Often development stops at leptotene.
2. With restitution nuclei.	Such plasmodia are rare. Different chromosome plates often have different chromosome numbers and lie oriented in different planes.

II. *No divisions.*

A.	Resting cells that never undergo division.
B.	Fusion plasmodia without attempts at nuclear division.

The formation of plasmodia is a characteristic feature of the apomictic species of *Calamagrostis* investigated by me (1946). On the female side the disturbances were not so varying as on the male side, but an important fact was that here there appeared the mitotical type of division, characteristic of many apomictic genera, among others *Calamagrostis*.

3. THE F_2 PLANTS.

Thirty-six plants have been investigated. The material was too small for studies in the remaining plants. As the individuals differ in some respects from each other, it is my intention first to give an account of every individual plant and then to sum up the facts in a table. The data are based on an investigation of at least 200 spikelets in every number.

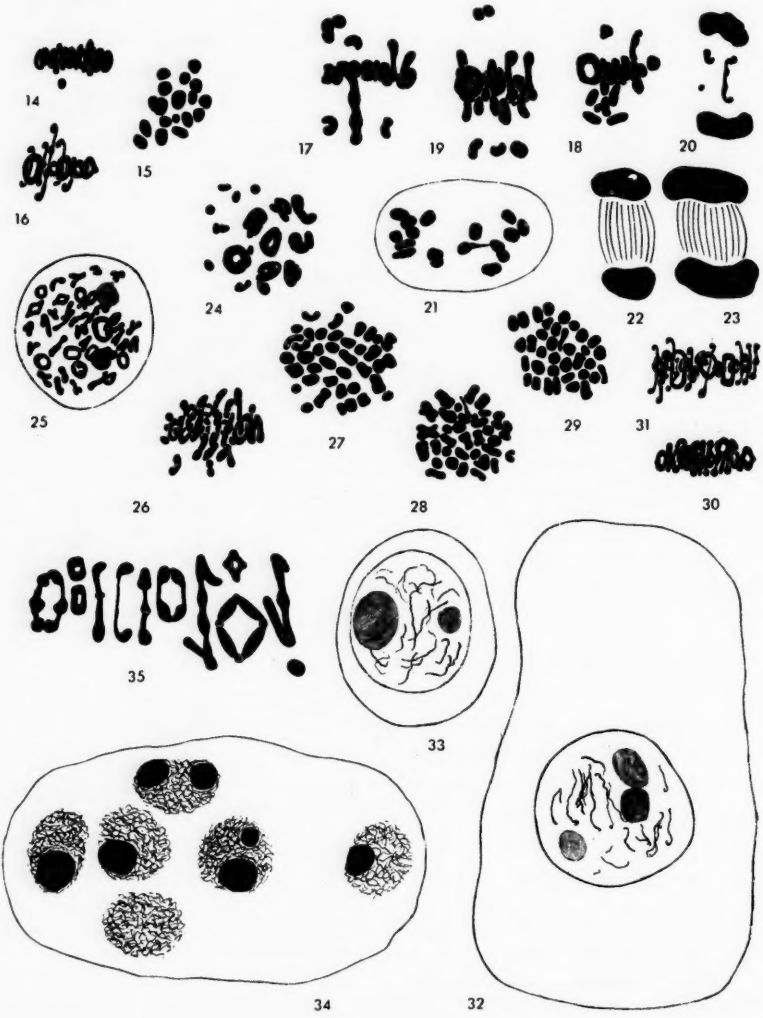
201—1. $2n = 56$. — 50 P.M.C.'s were studied. Thirty-four showed 28 II, six had 1 IV + 26 II, and in the remaining 10 P.M.C.'s the combination 1 III + 26 II + I occurred. On the female side the divisions were quite as regular as on the male side.

201—2. $2n = 73$. — No divisions could be ascertained on the male side, there being only plasmodia. E.M.C.'s with meiotic divisions have been observed.

201—4. $2n = 65$. — The divisions are regular on the male as well as on the female side. In the P.M.C.'s the most common configuration is 31 II + 1 III. In one case there have been observed 1 VI + 2 IV + 25 II + I. In 54 investigated P.M.C.'s the following distribution of quadrivalents and univalents could be observed.

Number of univalents				*Number of quadrivalents*		
0	1	2	3	0	1	2
40	6	4	4 = 54	43	9	2 = 54

In two of the P.M.C.'s having one quadrivalent a hexavalent ring was observed.



Figs. 14—35. 14—16, 201—7, 2n = 35. — 14, 17 II + I. — 15, 17 II + I. — 16, IV + + III + 14 II. — 17—23, 201—46, 2n = 37. — 17, IV + III + 12 II + 6 I. — 18, 2 IV + + III + 10 II + 6 I. — 19, 4 IV + 8 II + 5 I. — 20, Inversion bridge + fragment. — 21, first anaphase. — 22, first telophase, reduced. — 23, first telophase, unreduced. — 24, 201—21, 2n = 39, 8 IV + 7 I. — 25—28, 201—23, 2n = 72. — 25, diakinesis, IV + 34 II. — 26, first metaphase. — 27, IV + 2 III + 26 II + 10 I. — 28, 4 IV + + 3 III + 22 II + 3 I. — 29, 201—6, 2n = 72. — 30—31, 201—1, 2n = 56. — 30, bi-

201—5. $2n = 51$. — In the P.M.C.'s there are no divisions. As a rule they remain uninucleate resting cells. Sometimes the walls between the P.M.C.'s are gradually dissolved, and the whole loculus forms a plasmodium. During the long »resting stage» the P.M.C.'s swell very much, but to a different degree in various cells. The behaviour of the P.M.C.'s is exactly the same as in the apomictic species investigated. More than 300 spikelets have been studied. On the female side no safe observations were made.

201—6. $2n = 72$. — 51 P.M.C.'s have been studied. In 48 there occurred only bivalents and univalents, whereas in the 3 remaining P.M.C.'s a single quadrivalent was observed as well. The distribution of univalents appears from the following table.

Number of univalents

0	1	2	3	4	5	6
10	12	15	8	5	0	1 = 51

Some P.M.C.'s form resting cells. In three loculi a mixture of P.M.C.'s at different meiotical stages and resting cells occurred. An investigation of 147 cells gave 126 resting ones, while in 21 cells different meiotic stages were at hand. In a great number of loculi no divisions occur at all. On the female side meiotic as well as mitotic divisions were observed. In three out of nine E.M.C.'s investigated the latter division-type has been found.

201—7. $2n = 35$. — On the male side meiosis is very regular. 68 P.M.C.'s were analysed. In 36 there occurred 16 II + 1 III. In 29 of the 32 remaining cells a varying number of univalents was found.

Number of univalents

0	1	2	3
3	20	7	2 = 32

In all, only 5 quadrivalents were observed in these 68 P.M.C.'s. The female side showed no disturbances which were not already noted on the male side.

201—8. $2n = 71$. — A mixture of uninucleate resting cells and P.M.C.'s going through meiosis is present in the loculi. As a rule whole loculi degenerate, but sometimes a single cell in division can be found among other degenerated ones. In about 1.000 loculi investigated only 30 P.M.C.'s at meiosis were observed. In 15 of them there occurred quadrivalents besides bivalents and univalents.

Number of univalents

0	1	2	3	4	5
6	6	8	4	4	2 = 30

Number of quadrivalents

0	1	2	3
15	4	6	5 = 30

On the female side only meiotical divisions have been found.

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valents only. — 31, 2 IV + 24 II. — 32—33, 201—2, $2n = 73$. — 32, mitotic resting stage on the female side. — 33, resting stage on the male side. P.M.C.'s side by side at late meiosis. — 34, 201—24, $2n = 71$, pollen-plasmodium. — 35, 201—38, $2n = 62$, different configurations. — Magnif. 1100 ×.

201—9. 2n = 38. — In some loculi, resting cells occur side by side with normal tetrads. In most cases, however, the P.M.C.'s pass through a rather regular meiosis. An investigation of 212 P.M.C.'s at M I gave the following distributions:

19 II	17 II + III + I	18 II + 2 I	2 IV + 15 II	IV + 17 II	
170	32	4	2	4	= 212

At anaphase I some split bivalents remain in the equatorial plane instead of being distributed to the poles, which involves a high percentage of »univalents» during this stage.

Number of univalents

0	1	2	3	4	5	6	7
85	35	27	13	14	7	5	1 = 187

Only meiotic divisions were observed on the female side.

201—11. 2n = 51. — On the male side no meiosis at all occurs. P.M.C.'s form plasmodia, later on they degenerate. On the female side there occur meiotic as well as mitotic divisions. One mitotic division was found in 12 E.M.C.'s investigated. In one nucellus two E.M.C.'s lay close to each other. This has not been observed earlier in *Calamagrostis*, the archespore in this genus being unicelled in all the cases hitherto investigated.

201—12. 2n = 71. — The degeneration of the P.M.C.'s begins even at a very early stage. In some cases the cell walls are dissolved and plasmodia are formed of whole loculi. In 1680 loculi investigated not a single P.M.C. passing through meiosis was to be found. 2 out of 23 E.M.C.'s investigated showed mitotic divisions.

201—13. 2n = 37. — The plant lacks the capacity of forming pollen. Only plasmodia have been observed in 920 loculi. All E.M.C.'s investigated show meiotic tendencies, but, as fixations of later stages have not been studied, it cannot with any certainty be claimed that the mitotic division does not occur in this plant.

201—14. 2n = 71. — Principally there occur plasmodia and swollen P.M.C.'s — resting stages. In about one tenth of the investigated loculi, divisions were found — mainly the type described by LEVAN (1936—37, p. 98) as monokinetic meiosis, which is characterized by a complete loss of the second division. Dyads instead of tetrads are formed. In some cases tetrads have been found; but either they were very rare, or they did not separate but hung together. An investigation was carried out on 1050 loculi, and in 702 of these only plasmodia were to be found, while, in the 348 remaining loculi, dyads or tetrads were present. In the P.M.C.'s studied, only 2 cells could be completely analysed. In one cell there were 35 II + I, and in the other 32 II + 7 I. Trivalents are common, but no quadrivalents have been observed. The frequency of univalents was determined in 35 cells.

Number of univalents

0	1	2	3	4	5	6	7	8	9
0	4	6	17	15	5	3	2	0	1 = 53

On the female side only meiotic stages have been observed.

201—15. 2n = 36. — In some P.M.C.'s we find meiosis, in others resting stages. Plasmodium-containing loculi have been observed. When meiosis is at hand,

it develops, as a rule, regularly. The number of univalents and quadrivalents was determined in 62 cells.

Number of univalents					*Number of quadrivalents*			
0	1	2	3	4	0	1	2	3
38	12	8	3	1 = 62	57	4	1	0 = 62

18 II was the most common configuration. On the female side there is also meiosis.

201—17. 2n = 37. — Meiosis occurs on the male as well as on the female side. In some loculi plasmodia and resting cells have been found. The most common configuration in the P.M.C.'s is 18 II + I. The number of univalents and quadrivalents has been counted in 58 P.M.C.'s.

Number of univalents					*Number of quadrivalents*			
0	1	2	3	4	0	1	2	3
29	14	8	6	1 = 58	55	1	1	1 = 58

The distribution of chromosomes was determined in three cases at the second metaphase, 16—21, 17—20, and 18—19 being found.

201—18. 2n = 37. — Meiosis occurs on the male as well as on the female side. Multivalents are rare in the P.M.C.'s, only solitary trivalents having been found. The most common configurations analysed are 18 II + 1 I and 17 II + 3 I. The number of univalents was investigated in 150 P.M.C.'s.

Number of univalents									
0	1	2	3	4	5	6	7	8	9
8	63	28	30	7	10	1	1	1	1 = 150

201—19. 2n = 35. — The plant seems to lack any capacity of forming pollen. Only degenerated resting stages lie in the loculi, but no plasmodia have been observed. All E.M.C.'s investigated show meiosis, but no studies have been made in later stages.

201—20. 2n = 35. — In some loculi plasmodia occur; for the rest a rather regular meiosis was observed in the P.M.C.'s. The most common configurations noticed are 16 II + 1 III and 17 II + 1 I. In no case has more than 1 univalent/P.M.C. been found. The frequency was 8 cells with 1 univalent out of 58 counted. In 5 of these 58 cells a single quadrivalent occurred. On the female side only meiotic divisions have been observed.

201—21. 2n = 39. — On the male side the meiosis is much disturbed, but neither plasmodia nor resting cells have been found. In five cells the configurations were interpreted as follows:

1 III + 17 II + 2 I
1 IV + 1 III + 14 II + 4 I
19 II + 1 I
4 IV + 2 III + 8 II + I
8 IV + 7 I

The number of quadrivalents and univalents were determined in 52 P.M.C.'s.

Number of univalents							*Number of quadrivalents*				
0	1	2	3	4	5	6	0	1	2	3	4
12	17	10	3	8	1	1 = 52	20	11	7	11	3 = 52

On the female side one could observe meiotic divisions.

201—22. 2n = 37. — In the P.M.C.'s solitary trivalents occur; for the rest only bivalents and univalents are at hand. Succedent and simultaneous divisions vary, and for that reason there occur T-tetrads, range-tetrads and square-formed ones mixed in the loculi. The frequency of univalents was determined in 163 P.M.C.'s.

Number of univalents

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	27	26	31	28	23	9	9	2	2	1	1	0	0	1 = 163

On the female side only meiotic divisions were observed.

201—23. 2n = 72. — This type is remarkably regular in spite of the high chromosome number. On the male side, as a rule, some quadrivalents and univalents occur, otherwise only bivalents are present. At metaphase, 36 II have been observed once, just as the distribution 36—36 has been found at anaphase I. The most irregular configuration analysed at M I was 25 II + 22 I. At A I one could notice the distributions 35—37, 34—38, and 33—39. The number of univalents was counted in 35 P.M.C.'s and the number of quadrivalents in 30.

Number of univalents								*Number of quadrivalents*					
0	1	2	3	4	5	6	7	0	1	2	3	4	5
3	11	4	8	2	2	2	3 = 35	5	8	4	8	4	1 = 30

On the female side only meiosis has been observed, the numbers of multivalents and univalents are about the same as on the male side.

201—24. 2n = 71. — On the male side plasmodia are very common. Meiotic stages have only been observed in 3 P.M.C.'s out of 560 loculi investigated. These three cells showed regular meiosis and had 1 or 2 univalents, respectively; and for the rest only bivalents or bivalents + 1 trivalent. On the female side 35 II + I were observed in one E.M.C. No mitotic divisions were to be found.

201—26. 2n = 37. — On the male side the meiosis is rather regular with an abundance of bivalents. The monokinetic meiosis of LEVAN (1936—37, p. 89) was found in some loculi. The frequency of quadrivalents and univalents were investigated in 53 P.M.C.'s.

Number of univalents						*Number of quadrivalents*				
0	1	2	3	4	5	0	1	2	3	
33	5	2	8	4	1 = 53	44	5	0	4 = 53	

The distribution of chromosomes was examined in 23 cells at second metaphase.

Distribution of chromosomes at M II

16—21	17—20	18—19	
3	5	15	= 23

On the female side only meiotic divisions were observed.

201—27. 2n = 60. — On the male side plasmodia as well as meiotical divisions occur. 60 out of 116 loculi investigated only developed plasmodia, 52 had pollen only, and the 4 remaining had plasmodia as well as pollen. The meiosis is rather regular, only some quadrivalents and trivalents were observed.

Number of univalents						*Number of quadrivalents*				
0	1	2	3	4	5	0	1	2	3	4
40	8	3	1	0	1 = 53	36	9	4	2	2 = 53

All E.M.C.'s investigated show meiotic divisions.

201—28. 2n = 36. — On the male side principally plasmodia were found. When meiosis occurs the divisions are very irregular with great numbers of univalents and up to 5 quadrivalents. As a rule, there are no trivalents. Dyads and restitution nuclei are common. In the loculi P.M.C.'s at prophase stages often lie close to tetrads. In three cases it was possible to analyse the configurations completely: 5 IV + 5 II + 6 I, 2 IV + 11 II + 6 I, and 3 IV + 8 II + 8 I. The numbers of univalents and quadrivalents were counted in 51 cells.

Number of univalents											*Number of quadrivalents*				
0	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5
1	4	9	9	10	7	5	3	1	1	1 = 51	4	15	18	11	3 = 51

The distribution of chromosomes at M II was determined in 4 cases, 19—17 being found twice, 21—15 and 23—13 once. On the female side meiosis was observed to be irregular, but no mitotic divisions were found.

201—29. 2n = 36. — On the male side only plasmodia have been observed. On the female side meiotical divisions occur; they are often retarded, however, which might signify their having mitotic tendencies.

201—30. 2n = 38. — In about 50 % of the P.M.C.'s investigated no divisions were observed, all cells lying in resting stages and later on degenerating. The divisions are very regular when they occur; in most cases 19 bivalents are predominant. In 61 cells investigated 1 univalent was observed eight times and 2 univalents twice. No quadrivalents were noticed at all. In one case an inversion bridge was found. On the female side only meiosis was observed.

201—31. 2n = 36. — P.M.C.'s with meiotical divisions occurred only in one out of about 560 loculi investigated. In all other loculi resting cells were found, which degenerated sooner or later. In the loculus with divisions two cells at M I had the configuration of 17 II + 2 I. One P.M.C. at A I had an inversion bridge. Only meiotic divisions were determined with certainty on the female side.

201—35. 2n = 58. — Plasmodia, resting cells and P.M.C.'s with different meiotic divisions have been found on the male side. Bivalents are frequent, trivalents very rare, and quadrivalents have only been observed in 5 cells out of 70 investigated. The number of univalents was determined in 70 P.M.C.'s.

Number of univalents							*Number of quadrivalents*	
0	1	2	3	4	5	6	0	1
26	18	13	3	6	3	1 = 70	65	5 = 70

In three P.M.C.'s inversion bridges were observed. The female side has not been studied.

201—37. 2n = 35. — On the male side plasmodia, resting cells, and P.M.C.'s with meiotic divisions alternate in the loculi. Single trivalents are common, but quadrivalents rare. In one exceptional case, however, there was observed 2 IV + + 1 III + 10 II + 4 I. In most P.M.C.'s the configuration 16 II + 1 III alternates with 17 II + I. The number of univalents were determined in 50 loculi in order to obtain material for a comparison with a single loculus that had a very high percentage of univalents.

Frequency of univalents in 50 loculi

0	1	2	3	4
110	321	82	11	10 = 534

Frequency of univalents in a single loculus

0	1	2	3	4	5	6	7
0	5	8	10	7	0	1	2 = 33

In the single loculus the average of univalents is 3,0/P.M.C.'s, whereas the average of the 534 cells gives the value of 1,06. On the female side only meiotic E.M.C.'s have been observed.

201—38. 2n = 62. — Out of 560 loculi investigated there occurred tetrads in two, different meiotical divisions in one, and for the rest only resting cells. The P.M.C.'s show tendencies towards the mitotical division, which has earlier been observed in *Calamagrostis purpurea* (NYGREN, 1946, p. 160). The tetrads do not separate, just as the situation was in No. 201—14. In the loculus with meiotical divisions these were rather good, which allowed the configurations to be interpreted in 10 P.M.C.'s.

31 II	observed	3 times	6 IV + 19 II	observed	once
30 II + 2 I	»	once	III + 29 II + I	»	»
29 II + 4 I	»	»	VI + 2 IV + 2 III + 21 II	»	»
IV + 29 II	»	»			

Two hexavalents were found in all. On the female side mitotic-like resting stages were observed.

201—39. 2n = 58. — This plant has a rather regular meiosis on the male as well as on the female side. In the loculi the most common configurations are 29 II and 27 II + III + I. In some cases 28 II + 2 I were observed. The most irregular configuration analysed was 25 II + III + 5 I. Only 3 single quadrivalents have been observed in 67 cells analysed. It seems as if they were amphibivalents (HÅKANSSON, 1931, p. 29). The univalents were counted in 67 cells.

Number of univalents

0	1	2	3	4	5
23	25	10	6	2	1 = 67

On the female side only meiotical divisions have been noticed.

201—40. 2n = 71. — P.M.C.'s with meiotical divisions and degenerated resting cells alternate in the loculi. Meiosis is very regular with 34 II + III as the most

common configuration. Quadrivalents were rare in spite of the high chromosome number. The number of univalents and quadrivalents were determined in 53 cells.

Number of univalents					*Number of quadrivalents*			
0	1	2	3	4	0	1	2	3
33	10	3	5	2 = 53	47	4	1	1 = 53

Meiosis also occurs on the female side. In one single E.M.C. there could be observed the mitotic division type.

201—42. 2n = 38. — On the male side there are plasmodia in almost all loculi. When meiosis occurs in the P.M.C.'s it is, as a rule, very irregular, which can be seen from the great number of univalents.

Number of univalents												*Number of quadrivalents*	
0	1	2	3	4	5	6	7	8	9	10	11	0	1
3	7	11	10	6	6	3	2	0	1	0	1 = 50	47	3 = 50

On the female side irregular meiosis was observed in most E.M.C.'s, but in three cases the mitotic type of division was found.

201—43. 2n = 40. — A regular meiosis was noticed on the male as well as on the female side. On the male side trivalents occur in a rather great number of the P.M.C.'s. The number of observed quadrivalents, however, is very small. Only 7 single ones were found in a total number of 53 cells. The number of univalents is rather high, which probably stands in connection with the high frequency of trivalents.

Number of univalents					
0	1	2	3	4	5
5	20	13	9	5	1 = 53

On the female side it has only been possible to find meiotical stages.

201—45. 2n = ?. — No divisions at all were observed on the male side. The P.M.C.'s in the loculi degenerate sooner or later in some way or other. On the female side the mitotic type of division was found in three E.M.C.'s.

201—46. 2n = 37. — Meiosis is very irregular on the male side. Univalents or quadrivalents occur in every P.M.C. examined. Semiheterotypic division (ROSENBERG, 1926—27) was noticed in three P.M.C.'s. Unreduced gametes are rather common. The configuration 4 IV + 8 II + 5 I has been observed twice.

Number of univalents											*Number of quadrivalents*						
0	1	2	3	4	5	6	7	8	9	10	0	1	2	3	4	5	6
9	19	21	25	13	11	5	3	0	0	1 = 107	19	22	14	23	21	6	2 = 107

On the female side only meiotical stages were met with.

The results are summed up in Table 5.

TABLE 5. *The development of meiosis in the F_2 of the cross Calamagrostis arundinacea × epigeios.*

Plant No.	2n	Male side				Female side. Mitotic div.
		Regul. meiosis	Irregul. meiosis	Rest. st. or plasm. only	Rest. st. or plasm. + meiosis	
201 —1	56	x				
2	73			x		
4	65	x				
5	51			x		
6	72	x			x	x
7	35	x				
8	71		x		x	
9	38	x			(x)	
11	51			x		x
12	71			x		x
13	37			x		
14	71		(x)		x	
15	36	x			x	
17	37	x			(x)	
18	37	x				
19	35				x	
20	35	x			(x)	
21	39		x			
22	37		x			
23	72	x				
24	71	(x)			x	
26	37	x				
27	60				x	
28	36		x		x	
29	36				x	
30	38	x			x	
31	36	(x)			x	
35	58		x		x	
37	35	x			x	
38	62	(x)			x	x
39	58	x				
40	71	x			x	x
42	38		(x)		x	x
43	40		x			
45	—				x	x
46	37		x			

Rest. st. = resting stages; plasm. = plasmodia. — Only plants with remarkable irregularities have been included in the group »irregular meiosis». Thus, plants with »regular meiosis» may have one or two univalents.

The F_1 plant had plasmodia or resting stages in every loculus investigated, and a percentage of morphologically good pollen which lay

below 0,5. In F_2 no fewer than 25 plants or 69,4 % have either plasmodia or resting stages on the male side, and 5 out of these 25 plants did not develop any meiotical stages at all. Eighteen plants or 50 %, however, exhibited rather regular meiosis. On the female side the mitotic division has been found in 7 plants, or 20 % — a rather high number, as this type of division is very difficult to find in such plants as principally have meiotical divisions on the female side. Thus, it is very probable that it also occurs in some of the other F_2 plants. In many cases a regular meiosis on the male as well as on the female side in the F_2 plants makes a stabilization of the chromosome numbers in later generations plausible even on other chromosomal levels than the usual ones (i. e. 28, 42, 56). It seems quite possible that some of the F_2 plants with $2n = 70$, or thereabouts, would result in offspring having mainly this number. In the F_1 plant with $2n = 42$ the mitotic division gave embryo-sacs with egg-cells which needed fertilization in order to develop. In the cross *C. canescens* ($2n = 28$) × *C. epigeios* ($2n = 42$), which yielded hybrids with $2n = 56$, the egg-cells in the latter develop autonomously (NYGREN, 1948). On account of this, it is not possible to predict how the actual F_2 plants will behave in this respect. As there are at least four different chromosome numbers represented among the plants with the mitotical divisions stated, it will be an interesting task to find out whether the chromosome number *per se* plays any rôle in the autonomous development of the egg-cell.

III. TRANSGRESSION EFFECT IN A CROSS BETWEEN CALAMAGROSTIS PSEUDOPHRAGMITES AND C. VILLOSA.

A cross between *Calamagrostis pseudophragmites* (HALL.) BAUMG. and *C. villosa* (CHAIX.) GMEL. was made in 1944. In the summer of 1946 two of the hybrid plants obtained flowered. It was easy to establish that they looked very like *C. pseudophragmites*, but had much longer awns than this species. As modifications are very profound in *Calamagrostis*, the exceptional awn-length was regarded as being due to external circumstances. However, as in the summer of 1947 the same phenomenon was repeated, the reason could hardly be phenotypical. A closer study showed that the awns in the hybrids were fixed to the middle of the lemma, whereas *C. pseudophragmites* always has them fastened on the top of the lemma. *C. villosa* commonly has a very short awn fixed to the back of the lemma near or somewhat below the

middle (cf. HEGI, 1908, p. 232; ASCHERSON and GRAEBNER, 1898—1902, p. 203); but in this special case the father plant belonged to var. *mutica* TORGES and had no awn at all. In *C. pseudophragmites* the two glumes of the spikelet (one-flowered in *Calamagrostis*) are of different lengths, while in *C. villosa* they are equally long. In the hybrid the length is slightly different in some spikelets, but in others they look quite the same as in *C. villosa*.

Values were worked out in order to determine the variations in the parents regarding the length of the ligula, the lengths of glumes, and the length and fixing point of the awn. The mother plant, *C. villosa*, was rather constant. The length of the ligula was examined on the uppermost lamina of every straw of the plants, while the longest glume of 20 spikelets from different parts of the same panicle were measured. The lengths of awns and glumes were measured on the same spikelets. The values are to be found in Tables 6 and 7.

TABLE 6. *Length of ligulae and glumes in Calamagrostis pseudophragmites and C. villosa and their hybrids.*

Species	Length of ligula	Length of glumes								M
		40	45	50	55	60	65	70	75	(mm)
C. v.	3,8	2	4	11	2		1			5,05
C. ps. —46	8,0			4	6		9	1		5,78
» —47	6,5						9	4	7	6,52
C. ps. × *v.*, plant 1	7,5		1	19						5,01
» » 2	7,0						15	4	1	6,21
» » 3	6,5		4	14	2					5,01

Length of ligula in mm, of glumes in tenths of mm. — *C. ps.* = *Calamagrostis pseudophragmites*, *C. v.* = *C. villosa*, *C. ps.* × *C. v.* = the hybrid.

TABLE 7. *Length and form of the awn in Calamagrostis pseudophragmites and C. villosa and their hybrids.*

Species	Presence of awns	Fixing point of awn	Length of awn										M	Awn-form
			10	15	20	25	30	35	40	45	50	55	60	(mm)	
C. v.	0														
C. ps. —46	+	In the top		1	15	4								2,09	Str.
» —47	+	» » »	1	1	14	4								2,20	»
C. ps. × *v.*, pl. 1	+	At or above the middle					2	3	13	2				3,95	»
» » 2	+	At or below the middle									15	5		5,25	»
» » 3	+	At the middle							15	5				4,28	»

Length of awn in tenths of mm. — *C. ps.* = *Calamagrostis pseudophragmites*, *C. v.* = *Calamagrostis villosa*, *C. ps.* × *v.* = the hybrid. The awn is fixed on the lemma.

The most interesting fact in these hybrids is the transgression concerning the length of the awns. Transgressions having reference to different qualitative and quantitative properties are well-known in cereals and depend on cumulatively multiple alleles (cf. NILSSON-EHLE, 1909). The most probable explanation is that the transgression depends on complementary factors, but this involves the very important presupposition that complementary factors must be present in two *different* species of the same genus.

There are in *Calamagrostis* some other cases which may be interpreted in the same way as the one mentioned above. In two different crosses between *Calamagrostis canescens* (WEBER) ROTH and *C. neglecta* (EHRH.) there appeared in the F_2 plants with a very marked tufted manner of growth, in spite of both parents having no predisposition to this, their stolons being very well developed. In the F_2 of the cross *Calamagrostis epigeios* × *C. neglecta* a plant has segregated with branched straws, a characteristic feature of the species *C. canescens* and *C. purpurea* (TRIN.) TRIN., but never present either in *C. epigeios* or in *C. neglecta.*

IV. DISCUSSION.

In 1946 the present writer showed that hybrids between different species of *Calamagrostis* on the 28-chromosomal level had regular pairing at the first metaphase on the male as well as on the female side (NYGREN, 1946, p. 238). At that time only six combinations of hybrids had been investigated — and besides that, in most cases, only one or two combinations of each hybrid. During the two last years most mutual hybrid combinations between the Middle-European *Calamagrostis* species *pseudophragmites*, *varia*, and *villosa* have been studied, as well as the hybrids between these species and the Scandinavian species *arundinacea*, *canescens*, *epigeios*, and *neglecta* investigated earlier. It has been possible, of course, to find minor disturbances in the form of univalents and solitary multivalents of these hybrids, but such irregularities also occur in the pure species. Thus, meiosis must be regarded as regular in all the cases investigated. A hybrid between *C. canescens* and *epigeios* forms an exceptional case, as, besides a regular meiosis with 14 bivalents, it develops plasmodia in most loculi (NYGREN, 1948). Measurements of morphologically good pollen in various tetraploid hybrids show that the percentage figures only lie somewhat lower

than those of the pure species (cf. NYGREN, 1946, p. 238). Summing up the data we find:

(1) The *Calamagrostis* species with 2n = 28 all belong to the same coenospecies (TURESSON, 1922).

(2) Each species forms an ecospecies (TURESSON, 1922). The limits of fertility between various species are vague, however (cf. TURESSON, 1929).

(3) The different ecospecies in the genus are very closely related to each other, different species may have mutual complementary factors.

(4) In a cross between two different species of *Calamagrostis* there have arisen plants with characteristic features of a third species.

(5) All investigated amphimictic species of *Calamagrostis* with the chromosome number 2n = 28 have a distribution from Central Asia to the Middle or the North of Europe. The only circumpolar species is *C. neglecta*, whereas *C. canescens* and *C. varia* have a somewhat more limited distribution than the other species, of which, e. g., *C. villosa*, *C. epigeios*, and *C. arundinacea* have a wide distribution in East Asia.

(6) The Middle-Asiatic species *C. obtusata* TRIN. corresponds to *C. chalybaea* FR. in Scandinavia. The former species is an amphimict having excellent pollen (2n = 28?); the latter, on the other hand, is an apomictic species (2n = 42) which never develops pollen. Both species are so similar to some derivatives of the hybrid *C. arundinacea* × *C. canescens* that it is not unlikely that the isolated Fennoscandian species has originated from this hybrid instead of from the *C. obtusata* population.

In the work of BABCOCK and STEBBINS on *Crepis* (1938) the authors explain the origin and development of the American species of the genus in such a way as to derive these (x = 11) from the 4- and 7-paired species of the Old World. Thus they should be amphidiploids or polyploid derivatives of the latter. In *Calamagrostis* the situation is not so complicated, since all species investigated belong to a 7-paired series. The sexual species that are allogamous regularly have 14 bivalents at first metaphase, multivalents forming exceptions. As just mentioned, the same is true of their hybrids on the 28-chromosomal level. On the 42-chromosomal level gross disturbances in the form of resting cells and plasmodia occur in the combinations *C. arundinacea* × *C. epigeios*, *C. canescens* × *C. epigeios*, and *C. epigeios* × *pseudophragmites* (NYGREN, 1948). On the other hand, the pure species *C. epigeios* itself

shows a very disturbed meiosis at this chromosome number (NYGREN, 1946, p. 142), while other combinations of the hybrid *C. arundinacea* × *C. epigeios* with 2n = 42 behave regularly.

The most adequate explanation of these paradoxical facts is that the pure species on the 28-chromosomal level are amphidiploids. This may be the reason for the similar behaviour of the chromosomes in the pure species and in the hybrids. The chromosomes in the two different genomes of the amphidiploids may be so closely related to each other that they may pair in a hybrid. In haploid rye LEVAN (1942) found bivalents, trivalents, and even some quadrivalents, in spite of the fact that the plant had only one haploid genome of 7 chromosomes. On the 42-chromosomal level gross disturbances occur, as a rule, in *Calamagrostis* hybrids between *C. epigeios* 2n = 56 and other amphimictic species. The reasons for this condition may be:

(1) The heterogeneity of the polyploid derivatives of the assumed amphidiploid. If the genomes of *C. epigeios* on the 28-chromosomal level are *AA BB*, the hexaploid may be *AAA BBB*, *AA BBBB*, or *AAAA BB*, etc., and the octoploid *AAAA BBBB*, *AAAAA BBB*, or *AAA BBBBB*, etc.

(2) There is a defective homology between the different genomes of the hexaploid hybrid. If *C. arundinacea* has the genome-formula *CC DD*, the actual hybrid may have *AA BB C D*, *A BBB C D*, or *AAA B C D*. In the first case it is possible to receive a regular first metaphase, as *A* has the chance of pairing with *B*, and *C* with *D*. In the two last cases, however, there are not less than three single genomes, *A*, *C*, and *D*, or *B*, *C*, and *D*; and, besides these, three *A* or three *B* genomes. This may be the reason for the break-down of the meiosis in the hexaploid hybrid.

Of course, more genetical data must be collected before the evidence pointing to the 28-chromosomal *Calamagrostis* species being amphidiploids can be regarded as valid. However, the writer desires to submit this hypothesis here, being quite aware that the same interpretation may be given as to many other critical plant genera, e. g., the genus *Salix*.

I am indebted to Professor A. MÜNTZING for his kindness in giving me working space at the Institute of Genetics of Svalöf, and to Professor Å. GUSTAFSSON for valuable discussion. Pecuniary assistance has been received from Statens naturvetenskapliga forskningsråd.

SUMMARY.

(1) In a cross between *Calamagrostis arundinacea* (L.) ROTH (2n = 28) and *C. epigeios* (L.) ROTH (2n = 56), with the former as mother, it has been possible to obtain a hybrid (2n = 42).

(2) The meiosis of the hybrid was much disturbed on the male side; the P.M.C.'s regularly formed resting cells and plasmodia as in the apomictic species investigated in the genus. On the female side the mitotic division, characteristic of apomictic *Calamagrostis* species, has been found.

(3) Forty-nine F_2 plants were raised, 43 of these flowering in the summer of 1947. Eight plants with the chromosome numbers 35, 37, and 38 showed very remarkable similarities to *C. varia* (SCHRAD.) HOST. (2n = 28).

(4) The cytological investigation of the F_2 plants has shown that 7 plants with the chromosome numbers 2n = 38, 51, 62, 71, and 72 have mitotic divisions on the female side.

(5) A transgression effect in the length of awns in a cross between *Calamagrostis pseudophragmites* (HALL. fil.) BAUMG. and *C. villosa* (CHAIX.) GMEL. makes it very probable that complementary factors are at hand in the two different species mentioned of the same genus.

(6) The author advances the hypothesis that the sexual species of *Calamagrostis* with 2n = 28 are amphidiploids.

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CHROMOSOME STUDIES IN THYMUS

I. SOMATIC CHROMOSOME NUMBERS WITH SPECIAL REFERENCE TO THE FENNOSCANDIAN FORMS

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I. INTRODUCTION.

THE genus *Thymus* is one of the few species-rich plant genera concerning which only quite preliminary cytological observations have been made. The first and so far the only exact determination of the chromosome number was published as late as 1942, when LÖVE and LÖVE (1942 a, p. 15) observed in Scanian material of *Thymus Serpyllum* the number 2n = 24. Before this, only the approximate number 2n = = ca. 20, as determined by NĚMEC (1925), was known in this species. Some time ago VAARAMA (1948) wrote a paper presenting among others 7 determinations of chromosome numbers in the genus *Thymus*. The numbers found by him are (2n) 28, 30, 42, 54 and 58. On the basis of these determinations he briefly concludes that polyploidy as well as aneuploidy has played a rôle in the evolution within the genus.

The morphologico-taxonomic analysis of the collective species *Thymus Serpyllum* in Fennoscandia, carried out by me not long ago (JALAS, 1947 b), gave direct rise to the present study, which accordingly forms a sequel to it. During this study several cases were encountered in which morphology alone proved insufficient to warrant definite taxonomical conclusions being drawn and in which the support of cytogenetical data seemed to be needed.

The cytological material at my disposal is as yet very small when considered in relation to the polymorphism of the genus. It seemed, however, to be of interest to take under consideration the preliminary results so far achieved, since they throw some light on certain questions concerning the polymorphism and phylogeny of the species.

II. MATERIAL AND METHODS.

The plant material for the present study comprises (1) specimens collected from natural habitats in 1946 and 1947 and cultivated in the

Botanical Garden of Helsinki University, (2) species cultivated in the Botanical Gardens of Helsinki and Uppsala Universities, and (3) seedlings grown from seed obtained from foreign Botanical Gardens. The exact origin of each specimen will be given in the special section devoted to that species. Chromosome number have been determined in 25 individual plants, which fall into 12 species and subspecies as follows (alphabetical order, in brackets the number of specimens studied):

alpestris TAUSCH ..... (1)
caespititius BROT. ..... (1)
comosus HEUFF. ..... (1)
Drucei RONN., em. JALAS ..... (3)
Dubjanskyi KLOK. et DES.-SHOST. ..... (1)
marschallianus WILLD. ..... (2)
pulegioides L. ssp. *eu-pulegioides* RONN. ..... (3)
» » » *montanus* W. K. ..... (1)
Serpyllum L.; MILL. ssp. *angustifolius* (PERS.) VOLLM. .. (8)
» » » » *tanaënsis* (HYL.) JALAS ..... (2)
vulgaris L. ..... (1)
zheguliensis KLOK. et DES.-SHOST. ..... (1)

Seedlings were studied only in those cases in which it was possible to determine them taxonomically, since it was often found that the names under which seed were obtained from the different gardens were erroneous. The plants studied are to be seen in the Botanical Garden of Helsinki University and as herbarium specimens made of them in my own collection.

All determinations of the chromosome numbers have been made from root-tip material. In addition to a few fixations of embryonal roots in the spring of 1947, the material consists of adventive roots of grown plants fixed in the autumn of 1947. Fixation was carried out according to the method of WEBBER. Before fixation the plants were kept in cold except in a few cases when the roots were fixed late in the autumn and early in the morning, the maximum temperature being + 5° C. It turned out that on October 17, when the last fixations were made and for several nights the temperature had already fallen under the freezing point, the roots had almost ceased to grow. In the plerome no cell divisions could be observed, while in the periblem a certain number of divisions were to be seen, although not so many as earlier in the autumn. The latest cell divisions were to be found in the few cell layers situated directly under the dermatogen. All fixations were made

at 7.30 a. m.—12.30 p. m. The time of fixation does not seem to have any effect on the number of dividing cells.

Root tips were embedded in the usual manner in chloroform and parowax, and were cut into 10 μ sections. The slides were stained with gentian violet. Crystallization of the stain during differentiation, which to begin with hindered the staining, was overcome by a long washing (60 min.) in water after the staining and a quick passage through abs. alcohol—clove oil (1 : 1) before the final differentiation in terpineol.

The figures have been drawn at table level with the aid of ABBE's camera lucida fitted with an apochromatic immersion objective $\times$ 100 and ocular $\times$ 20 (Zeiss). Hence the magnification is ca. 3500.

III. THE CHROMOSOME NUMBERS FOUND.

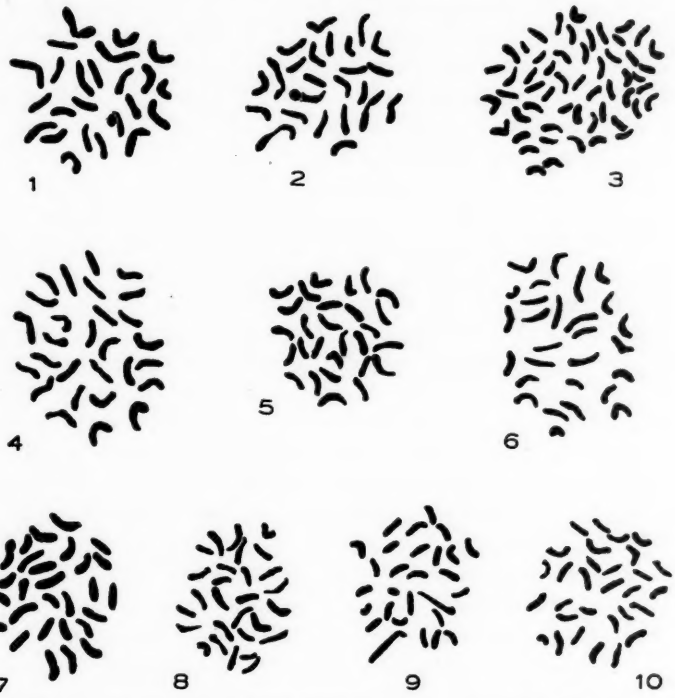
1. FENNOSCANDIAN THYMUS FORMS.

In Fennoscandia 6 species and subspecies of the genus *Thymus* are known at present (cf. JALAS, 1947 a and b). The chromosome number has been determined in all of them and the results are appended separately for each species. A brief account of its morphological delimitation and intraspecific variation is given for each species and subspecies, in so far as this seems to be necessary for the discussion of the results and their later utilization. The most important features in the general distribution of the forms are also described.

Th. vulgaris L. — Main distribution: Wild only in the areas around the western Mediterranean. Elsewhere commonly cultivated and only rarely gone wild (e. g., RONNIGER, 1944, p. 4). VAARAMA (1948) has found in this species the number $2n = 30$. The strain studied by him, which I have seen, is annual and belongs to the form *verticillatus* WILLK. et LANGE (WILLKOMM and LANGE, 1870, p. 403). I have counted the same chromosome number in an individual which had been raised from seed, obtained from Tashkend under the name of *Th. Serpyllum*. This strain did not flower during the growth period and thus seems to be perennial.

Th. marschallianus WILLD., s. lat. — A critical form-group which still needs a final analysis. It is distributed to the eastern parts of Central Europe and the Russian steppe regions. *Th. marschallianus* s. str. has occasionally been found in the eastern Fennoscandia in the provinces of Ka and Ik, whence it has been dispersed via the railway. One plant-individual belonging to this group (No. 51; cultivated in the Botanical

Garden of Helsinki University), which has been studied by me, resembles fairly closely the main type of *marschallianus*. In spite of several attempts I did not succeed in obtaining faultless metaphase plates, so the chromosome number is not quite certain. Most probably it is 2n = 28. The other individual studied (No. 56) has been cultivated



Figs. 1—10. Somatic metaphase plates from root tips. — 1: *Th. Serpyllum* ssp. *angustifolius* (No. 9). 2n = 24. — 2: *Th. Serpyllum* ssp. *tanaënsis* (No. 40). 2n = 24. — 3: *Th. Drucei* (No. 98). 2n = 54. — 4: *Th. pulegioides* ssp. *eu-pulegioides* (No. 44). 2n = 28. — 5: *Th. pulegioides* ssp. *montanus* (No. 76). 2n = 28. — 6: *Th. alpestris* (No. 68). 2n = 28. — 7: *Th. comosus* (No. 91). 2n = 28. — 8: *Th. marschallianus* (No. 56). 2n = 28. — 9: *Th. zheguliensis* (No. 54). 2n = 28. — 10: *Th. caespititius* (No. 66). 2n = 30.

in the Botanical Garden of Uppsala University under the name of *Th. Kotschyanus* BOISS. et HOHEN. and was raised from seed obtained from Erivan. It comes near the form *Th. latifolius* (BESS.) ANDRZ., which in my opinion is not more than a subspecies of *marschallianus* (cf. KLOKOV and SHOSTENKO, 1938, pp. 125—127). The diploid chromosome

number of this form is 28 (Fig. 8). More determinations are needed in this form-group.

Th. pulegioides L. — One of the most polymorphic species in the genus and one that may be divided into at least four subspecies. In Fennoscandia only the subspecies *eu-pulegioides* RONN. is found (RONNIGER apud CALLIER, 1927, p. 22. Syn. *Th. pulegioides* L. s. lat. RONNIGER, 1924, p. 323; *Th. pulegioides* L. I. ssp. *pulegioides* RONNIGER apud HAYEK, 1931, p. 369; *Th. pulegioides* L. I ssp. *genuinus* STOJANOFF and STEFANOFF, 1933, p. 893). This subspecies is purely European and its centre of variation seems to be in Central Europe, where numerous — in the main rather insignificant — varieties and forms have been distinguished within it (compare, e. g., RONNIGER, 1933 a, pp. 133—134). In Fennoscandia the subspecies is indigenous to certain regions. Several subforms that later spread from elsewhere to these areas have created in Sweden a form complex of the subspecies which, at least on morphological grounds, can hardly be subdivided into smaller taxonomical units (cf. HYLANDER, 1943, pp. 49—51). If we consider the main features and disregard certain southeastern forms, we may distinguish two form groups or, rather, tendencies which correspond — with numerous intermediates and uncertain cases — to the varieties *ovatus* (MILL.) RONN. and *Chamaedrys* (FR.) RONN., including the insignificant varieties around these (cf. RONNIGER, 1924, p. 325; 1933 a).

In this subspecies I have made the following three determinations:

No. 44 v. *ovatus* spont. Norway, Trondheim, leg. 1947 G. BRODAL (Fig. 4) ..... 2n = 28
No. 42 v. *Chamaedrys* cultivated in the Botanical Garden of Helsinki University under the name of *Th. Chamaedrys* v. *citriodorus* ..... 2n = 28
No. 23 v. *ovatus* raised from seed obtained from Antwerp under the name of *Th. Serpyllum* ..... 2n = 28

The same chromosome number has been found by VAARAMA (l. c.) in a specimen cultivated under the name of *Th. ovatus*. It may be mentioned that both the plants, No. 23 and No. 42, raised from seed, flowered already during their first period of growth.

Th. Drucei RONN., em. JALAS, emend. nova. — Syn. *Th. Serpyllum* L., em. FR. ssp. *arcticus* (DUR.) HYL., em. JALAS (JALAS, 1947 b, pp. 23—24, detailed account of synonyms and description; cf. also p. 52). The chromosome numbers found are:

No. 98 spont. Norway, Trondheim, leg. 1947 G. BRODAL (Fig. 3) ..... 2n = 54
No. 99 spont. Iceland, Ingolfsfjall, leg. 1947 A. LÖVE ..... 2n = 54
No. 61 unnamed specimen growing in the Botanical Garden of Helsinki University resembling the form *neglectus* RONN. ..... 2n = 54

Two of VAARAMA's (l. c.) determinations agree with the above-mentioned. One of the plants belongs to the thickly pubescent form, *Th. britannicus*, which is commonly cultivated in gardens. The other, which apparently represents the species now in question, bore the erroneous name *Th. Doerfleri* RONN.

The determinations indicate that the different forms of this form group probably have only a single chromosome number, 2n = 54, in the different parts of its distribution area. This number cannot be directly derived from the chromosome numbers of the two remaining subspecies of *Th. Serpyllum* (cf. below). This form group is consequently genetically isolated from *Th. Serpyllum*, since the different chromosome numbers make crossing impossible, or at least give rise to sterile hybrids. Since *Th. Drucei* fulfils in all respects the demands of modern taxonomy in regard to a species (HUXLEY, 1945, pp. 164—165), it is justified and even obligatory to give it the rank of an independent species. As was pointed out earlier by the present author (JALAS, l. c., p. 52), this measure is warranted even from a morphological viewpoint. The phylogeny and taxonomic position of the species will be dealt with below.

Th. Serpyllum L., em. MILL.[1] — After the delimitation of *Th. Drucei* from *Th. Serpyllum* as a separate species, the latter comprises two Fennoscandian subspecies, ssp. *angustifolius* (PERS.) VOLLM. and ssp. *tanaënsis* (HYL.) JALAS. For details as to their distribution and taxonomy, see JALAS, l. c. In order to find out whether differences exist in the chromosome complements of the two subspecies, and on the other hand between the varieties of ssp. *angustifolius* distinguished by me, I have studied several forms and samples of the subspecies from different parts of the Fennoscandian distribution areas (Fig. 11). The results are as follows:

[1] In my earlier paper on *Thymus* I have, in accordance with RONNIGER (e. g., 1924, 1933 a), given FRIES (1828) as the emendator of the species. In his latest paper RONNIGER (1944, p. 20) states that MILLER (1768) had already given the current definition of the species.

Ssp. *angustifolius*

No. 24 v. *linnaeanus* St, Köyliö, Kankaanpää, leg. 1946 L. O. ERVI ..... 2n = 24

No. 25 v. *linnaeanus* (not typical) N, parish of Tammisaari, Tvärminne, Storängsberget, leg. 1945 ipse ..... 2n = 24

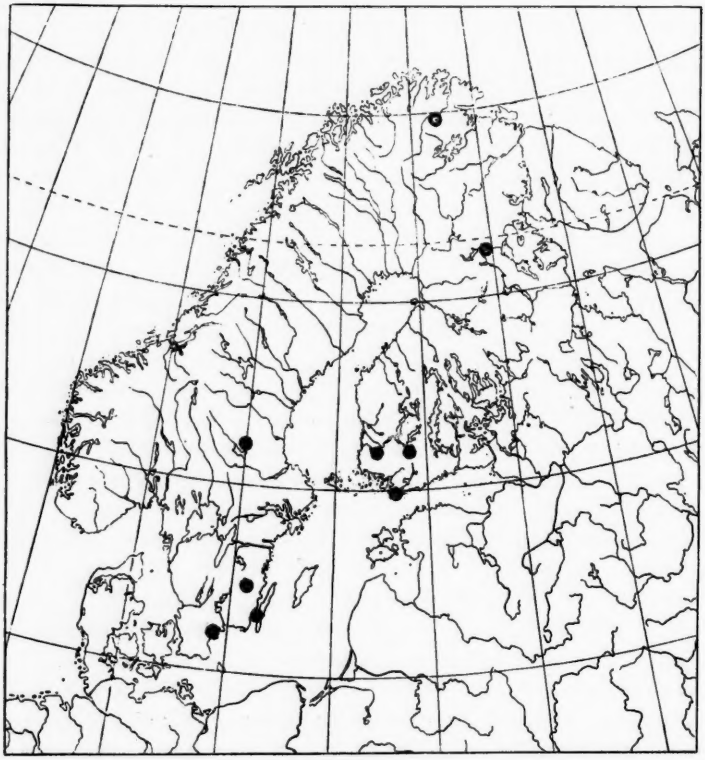


Fig. 11. The origin of the wild Fennoscandian *Thymus* forms studied. — ●, *Th. Serpyllum* ssp. *angustifolius*, 2n = 24. — ○, *Th. Serpyllum* ssp. *tanaënsis*, 2n = 24. — ×, *Th. Drucei*, 2n = 54 and *Th. pulegioides* ssp. *eu-pulegioides*, 2n = 28.

No. 13 v. *linnaeanus* f. *hirsutus* Småland, Virserum, leg. 1947 ipse ..... 2n = 24

No. 4 v. *ericoides* N, parish of Tammisaari, Syndalen (on a dune), leg. 1946 H. LUTHER ..... 2n = 24

No. 9 v. *ericoides* Öland, Resmo (on the alvar), leg. 1947 ipse (Fig. 1) ..... 2n = 24
No. 5 v. *rigidus* Ta, Hattula, Parola, leg. 1947 ipse ..... 2n = 24
No. 10 v. *rigidus* Småland, Järeda, Järnforsen, leg. 1947 ipse 2n = 24
No. 8 v. *rigidus* Dalarne, Ore, Furudal, leg. 1947 ipse ..... 2n = 24

Ssp. *tanaënsis*

No. 38 Ks, Kuusamo, Juuma, Jäkälävuoma, leg. 1947 ipse .. 2n = 24
No. 40 Li, Utsjoki, Mandojärvi, leg. 1947 T. H. SUOMINEN (Fig. 2) ..... 2n = 24

On the basis of its habitat (»dunes in Åhus») I should regard it as probable that the form of *Th. Serpyllum* studied by LÖVE and LÖVE (1942 a, p. 15) was ssp. *angustifolius* v. *ericoides*. Other forms of the species from this locality have not been found by me in the Scandinavian collections (JALAS, l. c., p. 67).

The above results indicate that at least in Fennoscandia *Th. Serpyllum* is a uniform species in regard to its chromosome number.

2. DETERMINATIONS IN NON-FENNOSCANDIAN THYMUS FORMS.

The original intention was to limit the study in this connection to the Fennoscandian forms, this because of the fact that the majority of the seedlings of the foreign species were still too small for the present purpose. Below, however, certain chromosome numbers found in non-Fennoscandian species are presented to give the following discussion a broader basis.

Th. caespititus BROT. — A clearly restricted and monotypic species, endemic to the western part of the Pyrenean peninsula (cf. WILLKOMM and LANGE, 1870, p. 404). Seed obtained from Kew Gardens. Chromosome number established as 2n = 30 (Fig. 10).

Th. Dubjanskyi KLOK. et DES.-SHOST. — Belongs to a group, consisting of several closely related forms, which is found in the southeastern parts of European Russia, in northern Caucasus and Dagestan (KLOKOV and SHOSTENKO, 1938, pp. 134, 138). The specimen now studied has been cultivated in the Botanical Garden of Uppsala University from seed obtained from Leningrad in 1939. The description of the Russian authors of this species (KLOKOV and SHOSTENKO, l. c., p. 138) applies well to this plant. It cannot thus belong to the species *Th. rariflorus* C. KOCH, in which RONNIGER (1933 b, p. 153) has included *Th. Dubjanskyi* as a variety. I have not been able to determine the exact chromosome number from my present material. It seems, however, to be > 50.

Th. zheguliensis KLOK. et. DES.-SHOST. — The species belongs to a group distributed to the middle Volga, the peripheral Ural Mountains and the northwestern areas of Central Asia (KLOKOV and SHOSTENKO, l. c., p. 132). The specimen studied by me has been cultivated in the Botanical Garden of Uppsala University from seed obtained from Kuibyschev University in 1940. Its chromosome number is 2n = 28 (Fig. 9).

Th. pulegioides L. ssp. *montanus* (W. K.) RONN. — Distribution area: Central European and Carpathian Mountains, northern parts of the Balkan. A deviating form is found in Georgia (RONNIGER, 1933 b, p. 155). Polymorphic (cf. RONNIGER apud HAYEK, 1931, pp. 371—373). There exist intermediate forms between it and ssp. *eu-pulegioides*. Seed has been obtained from the Botanical Garden of the Cluj University, the seed catalogue (1947) of which states the plant to have grown on a natural habitat. It flowered during the first growth period. The chromosome number is $2n = 28$ (Fig. 5).

Th. alpestris TAUSCH. — Distribution area: the Alps, the peripheral Alps, the Vosges Mountains, Schwarzwald, the Sudetic Mountains, Jauerling. As a relict in Thuringia, Wurtemburg, Baden, and Bohemia (RONNIGER, 1944, p. 12). The species is closely related to *Th. pulegioides*. The specimen studied by me has been cultivated under the name of *Th. marginatus* in the Botanical Garden of Uppsala University from seed obtained from Cluj in 1935. Its chromosome number is $2n = 28$ (Fig. 6).

Th. comosus HEUFF. — The species is native in southeastern Europe (Transylvania). The specimen growing in the Botanical Garden of Helsinki University showed the chromosome number $2n = 28$ (Fig. 7).

3. CERTAIN CHROMOSOME MORPHOLOGICAL OBSERVATIONS.

Dr. A. VAARAMA, in a letter to the present author, has expressed the opinion that the small size and relatively great number of chromosomes in the genus *Thymus* render chromosome morphological studies in this genus impossible. This is indeed true of several, perhaps most of the species. Certain cases in my material seem, however, to give cause for a closer comparison of the chromosome complements of the different species.

The species which have 28 somatic chromosomes, viz. *Th. pulegioides*, *Th. alpestris*, *Th. comosus* and *Th. marschallianus*, have rather similar chromosome complements. The different species cannot be distinguished by means of them. It is also impossible to decide with certainty which chromosomes in a plate form a pair, i. e. are homologous. The size differences of the chromosomes are too small for this, their length varying between 0,8—1,2 μ (cf. Figs. 4—8).

Th. zheguliensis, which also has $2n = 28$ chromosomes (Fig. 9), differs clearly from the previous group. It seems quite certain that this species has one chromosome pair with a subterminal centromere, which is clearly of bigger size, i. e. ca. 1,9—2,2 μ, than the other chromosomes. In addition the species has probably a very short chromosome pair. The case must be studied further, as these chromosomes have not always been possible to identify even in good metaphase plates. It is, however, certain that the chromosome complement of *Th. zheguliensis* is not identical with the other species with 28 chromosomes, mentioned above.

The two subspecies of *Th. Serpyllum*, viz. ssp. *angustifolius* and ssp. *tanaënsis*, seem to be similar in regard to their chromosome complements. The chromosomes in the basic chromosome set of the species are of the same size as in the *Th. pulegioides* group. *Th. Serpyllum*, however, in all cases studied has been observed to have four chromosomes that are twice the size of the others and that have median or submedian centromeres (Figs. 6 and 9). These four long chromosomes are in my opinion also to be seen in the Fig. 36 of LÖVE and LÖVE (1942 a, p. 13).

Th. Drucei deviates from all the other species studied in regard to the small size of its chromosomes (Fig. 3). In this respect it also differs clearly from the species which have the same chromosome number (*Th. pallasianus*, $2n = 54$) or a number close to it (*Th. longidens* v. *lanicaulis*, $2n = 58$; cf. VAARAMA, l. c., Figs. 11—13).

IV. DISCUSSION OF THE OBSERVATIONS.

1. NATURAL SUBUNITS IN THE GENUS THYMUS.

To obtain a survey of the facts available, I have set up a scheme comprising the most important taxonomic subsections of the genus and the determinations of the chromosome numbers for the different species and subspecies known for the present. The order of the different groups used here does not imply any definite opinion in regard to this question, my knowledge of the genus and the material available being too small for that. I have, however, tried to follow the probable course of the age of the different groups. It is evident that the branches of a genealogical tree, springing as they do in many directions, or parallel evolutionary series cannot be represented by a simple list. The division into sections and subsections has been made according to BRIQUET (1897, pp. 311—313). The subsection *Serpylla* has been classified mainly according to KLOKOV and SHOSTENKO (1938). It must be noticed that the last-mentioned authors have built up their system on the basis of Russian material and do not suppose it to be complete as such having reference to the *Thymus* flora of other and larger areas. The group *Marginati* KERN., which I was compelled to include in lack of a better classification, does not seem, at least as such, to be comparable with the groups of the Russian authors.

From the scheme it will be seen how much there still remains to be studied in the genus. A whole section and two subsections together

with several *Serpylla* subgroups not included in the scheme are wholly unstudied in regard to their chromosome numbers. All the chromosome numbers given are somatic (2n).

	2n	
Section I. PSEUDOTHYMBRA BENTH.		
Section II. SERPYLLUM BENTH.		
Subsection 1. Mastichinae BRIQ.		
Subsection 2. Vulgares BRIQ.		
Th. vulgaris L.	30	VAARAMA, 1948; JALAS
Th. × *citriodorus* PERS. (? = *pulegioides* × *vulgaris*)	42	VAARAMA, 1948
Subsection 3. Piperellae BRIQ.		
Th. caespititius BROT.	30	JALAS
Subsection 4. Bracteatae BRIQ.		
Subsection 5. Serpylla BRIQ.		
Subbracteati KLOK. et DES.-SHOST.		
Th. Dubjanskyi KLOK. et DES.-SHOST.	>50	JALAS
Th. pallasianus H. BRAUN	54	VAARAMA, 1948
Kotschyani KLOK. et DES.-SHOST.		
Th. zheguliensis KLOK. et DES.-SHOST.	28	JALAS
Verticillati KLOK. et DES.-SHOST.		
Th. marschallianus WILLD.	28	JALAS
Goniotrichi BORBAS		
Th. pulegioides L. ssp. *montanus* (W. K.) RONN.	28	JALAS
Th. pulegioides L. ssp. *eu-pulegioides* RONN.	28	VAARAMA, 1948; JALAS
Th. alpestris TAUSCH	28	JALAS
Marginati KERNER		
Th. comosus HEUFF.	28	JALAS
Eu-Serpyllum KLOK. et DES.-SHOST.		
Th. longidens VEL. v. *lanicaulis* RONN.	58	VAARAMA, 1948
Th. Drucei RONN.; JALAS	54	VAARAMA, 1948; JALAS
Th. Serpyllum L.; MILL. ssp. *tanaënsis* (HYL.) JALAS	24	JALAS
Th. Serpyllum L.: MILL., ssp. *angustifolius* (PERS.) VOLLM.	24	LÖVE and LÖVE, 1942 a; JALAS

2. VARIATION OF THE CHROMOSOME NUMBER IN A NATURAL TAXONOMIC UNIT.

Numerical changes in the chromosome complement give in different cases rise to taxonomical differences of different degree. On the one hand large plant groups are known, e. g. the Gymnosperms, in which a single chromosome number prevails, and on the other hand we have species which have several chromosome numbers, the different forms not being separable on taxonomical grounds (cf. DARLINGTON, 1937, pp. 227—228). These are extreme cases which are connected

through numerous intermediate types. The material available already gives some indications to which group the genus *Thymus* is to be included. Naturally we must not forget that further studies may bring to light facts which in some way or other will change the impression now received.

In four cases the chromosome determinations were made from two or more subspecies or forms of one species, e. g. *Th. vulgaris*, *Th. pulegioides*, *Th. Drucei*, and *Th. Serpyllum*. The result is in so far negative as the chromosome number was in no case found to vary within a single species. The advancement of *Th. Drucei* from a subspecies to the rank of an independent species may be regarded as a measure based on taxonomical data to which the observation of the chromosome number only gave the last impulse. In fact, this measure implies only the revival of an earlier view. Prof. K. LYKA, by his determinations, appears to have restricted and estimated the form group in question in the same manner. He has not, however, published his results (cf. JALAS, 1947 b, p. 53).

Two of the more thoroughly studied species mentioned above, viz. *Th. pulegioides* and *Th. Serpyllum*, belong to the most polymorphic representatives of the genus. Of these, subspecies as well as relatively insignificant taxonomical units were studied. It seems therefore most probable that the multiformity of the species is not accompanied by such changes in the genome as different chromosome numbers. In this respect the genus *Thymus* would seem to differ from another species-rich genus belonging to the family Labiatae, i. e. *Mentha*. In many species of this last-named genus, e. g. *Mentha arvensis*, already known in Scandinavia to be very polymorphic, several different chromosome races have been found (see LÖVE and LÖVE, 1942 b, p. 48).

Of *Th. Serpyllum* and *Th. Drucei*, both pubescent and glabrous forms have been studied. In this case, too, no difference in chromosome number has been found. This should be noted, since here we have a character which has been regarded as possessing considerable taxonomical value. Evolution has already proceeded so far in phylogenetically old species groups, according to RONNIGER (1927 a, p. 239), that the glabrous and pubescent forms may be regarded as separate species. Even their ranges of distribution often do not cover each other. This is the case in regard to the following pairs of species: *Th. marschallianus—Th. pannonicus* and *Th. pulegioides—Th. froelichianus*. The two latter members of these pairs are pubescent, and in these cases clearly more western as to their distribution than the glabrous ones.

RONNIGER (l. c.) regards the pubescent forms as more original than the glabrous ones. According to him, evolution in phylogenetically young form groups, e. g. within *Th. Serpyllum*, has not had time to lead to complete isolation of the strongly pubescent »race», and he suggests it to be distinguished »only» as a subspecies from the glabrous form group (cf. JALAS, 1947 b, pp. 40—41). It will be interesting to see whether the parallel species in the old form groups mentioned differ in regard to their chromosome complements. As mentioned above, in phylogenetically young species no differences in this respect have been observed.

The material is too restricted to allow anything to be said concerning the variation of the chromosome number in taxonomical units larger than species. The group *Goniotrichi* seems to be uniform in the light of recent studies. We must, however, remember that only two closely related species have been studied, and that the group is not clearly limited. It has, in fact, been formed on the basis of only one character, the pubescence of the stalk, and as early an observer as BORBAS (1892, p. 67) points out that a number of forms with *two-sidedly* hairy stalks might be more conveniently placed into other groups. Such forms connect the group *Goniotrichi* i. a. with the groups *Marginati* (*Th. carpaticus* ČELAK.) and *Eu-Serpyllum* [e. g., *Th. pseudochamaedrys* (H. BRAUN) RONN., *Th. alpigenus* KERN., *Th. longicaulis* PRESL. and also *Th. Drucei* RONN.; JALAS].

In two groups, *Subbracteati* and *Eu-Serpyllum*, chromosome numbers (54, 58) are known which may be derived from higher degrees of polyploidy. It must, however, be specially noted that no case of pure euploidy is known in which two or more members of one series are known. The high chromosome numbers found in the group *Subbracteati* are in accordance with the results of morphological taxonomy in that both the species studied are, according to KLOKOV and SHOSTENKO (1938, pp. 149, 150), relatively young members of their evolutionary series, *Th. Dubjanskyi* being even one of the two most specialized races in its series.

It seems to be a pertinent fact that of the six groups of the *Serpylla* subsection four have been found to have the same chromosome number, $2n = 28$, and especially that the chromosome complements of the species belonging to *Verticillati*, *Goniotrichi* and *Marginati* have not been found even morphologically to differ from each other. It seems possible that these chromosome complements are so identical that interspecific hybrids are fertile. This would partly explain the multiformity

of the species and the great number of intermediates. It may be mentioned that even recently certain species and varieties have on morphological grounds been supposed to be hybridogenous (e. g., RONNIGER, 1933 a, p. 132; 1944, p. 10). On the other hand, according to KLOKOV and SHOSTENKO (l. c., p. 152), hybridization does not play any part in the speciation of the genus *Thymus*. In my opinion hybridization must, however, in later studies be taken into consideration as a possible factor in the evolution.

In regard to the chromosome numbers in the different subsections the only fact known for the present is that both the subsections *Vulgares* and *Piperellae* have the same number, $2n = 30$. At least according to present knowledge, this number is absent in the subsection *Serpylla*. The possible bearing of this fact on the taxonomic differentiation of the genus is only determinable on the basis of further data.

The facts described above indicate that the genus *Thymus* in regard to the grouping of its chromosome numbers should belong to the same category as, e. g., *Viola* (CLAUSEN, 1927, 1929) and *Primula* (BRUUN, 1932) and several other plant genera to which, according to BRUUN (l. c.), the genus *Crepis* also belongs. They all have in common the feature that »a constant basic number prevails in the majority of groups, but at some points in the system this regularity is broken by the accretion of one or more chromosomes. Further, certain groups seem to show a greater inclination to polyploidy than others» (BRUUN, l. c., p. 206).

3. CERTAIN PHYLOGENETICAL ASPECTS IN THE SUBSECTION SERPYLLA BRIQ. THE BASIC CHROMOSOME NUMBERS OF THE GENUS.

According to our present knowledge, two different basic numbers are found in the subsection *Serpylla*, viz. 12 and 14, the former being represented by *Th. Serpyllum* alone and the latter being found in several groups of the subsection. Taxonomic data make it seem certain that the forms with these different numbers have a common origin. The question thus arises what the interrelation of these numbers is and how old phylogenetically the differences displayed by their genomes are.

Taxonomists agree in that *Th. Serpyllum* and its closest relatives are phylogenetically young species, being in many respects more specialized than, e. g., *Th. marschallianus* and *Th. pulegioides* (compare, e. g., RONNIGER, 1927 a, p. 238; KLOKOV and SHOSTENKO, 1938, p. 147). Arguing in favour of this opinion are many important morphological facts together with the distribution of the species. The latter species

are also young representatives of their groups, so the position is not to be interpreted to the effect that *Th. Serpyllum* is directly derivable from them. According to RONNIGER (1930, pp. 67—68), the northern *Eu-Serpyllum* forms may be traced back directly to the Mediterranean countries where certain relict-like species, *Th. nitidus* GUSS. (Sicily), *Th. Richardii* PERS. (the Balearic Islands; cf. KNOCHE, 1923, Tab. VI), *Th. Mariae* SENNEN (the eastern Pyrenees) and *Th. Willkommii* RONN. (the coastal mountains of Catalonia), are as the oldest and most primitive forms, being at the same time older and partly more primitive than the studied species belonging to the groups *Verticillati* and *Goniotrichi*.

If we take into consideration the specialization and youth of *Th. Serpyllum*, it is rather surprising that its chromosome number is, at least so far, the lowest one found, so much the more so as the forms in question are the most northern in regard to their distribution. We may therefore assume that the haploid chromosome number 12 of *Th. Serpyllum* might be secondarily derived from the number 14, which is much more common in the subsection. The reverse relation seems scarcely to be possible. The chromosome morphological data presented above also seem to agree with this assumption. It seems probable that four of the 14 haploid chromosomes of some ancestral *Serpyllum* form would have fused in two's, thus giving rise to a new secondary basic number 12. This would imply that these chromosomes had possessed a terminal centromere, in which case a »fusion» would have occurred. If the centromere had been subterminal a translocation would have taken place resulting in the formation of two big and two very small chromosomes. In that case we should have to suppose that the two smaller chromosomes would have disappeared, a common occurrence in the case of small fragment-like chromosomes. The final result has in any case been the formation of two big chromosomes provided with median or submedian centromeres.

If the observation made on *Th. zheguliensis*, viz. that it should have one long and one very short pair of chromosomes, is verified, it would support the latter alternative, i. e. translocation, since it would prove translocations to have occurred even later and in another line of the genus. *Th. zheguliensis* differs from *Th. Serpyllum* only in that even the shorter arms of the two chromosomes would form a successful combination in the former, the chromosome number remaining the same in spite of the translocation.

Another possibility of explaining the origin of the two long chromosome pairs in *Th. Serpyllum* is offered by the assumption that the

haploid chromosome number of the basic form had been 7. Instead of two fusions we should in this case have only one fusion or translocation, as described above, but on the other hand we should be compelled to operate with hypothetical ancestral forms having 6 or 7 chromosomes. Further, all the four long *Th. Serpyllum* chromosomes ought in that case to be homologous.

Changes in the basic chromosome numbers through fusion or translocation is a common occurrence in the plant kingdom, and according to BRUUN (1932, p. 162) »in the evolution of new karyological types this process must be assigned an important rôle». Very convincing cases of fusion have been met with in the genus *Crepis*. NAVASHIN has found them in nature (1931 a, pp. 202, 206) and produced them artificially with x-rays (1931 b, p. 251). In *Cardamine pratensis* in one case a chromosome number $2n = 30$ has been observed, which deviates from the known basic number (8). In the chromosome complement of this form there are two chromosome pairs twice the size of the other chromosomes; these are supposed to have arisen in the same manner as described above for *Th. Serpyllum* (LAWRENCE, 1931, pp. 185, 198). A comparison of the chromosome complements suggests the presence of similar changes in the genus *Matthiola*, where »in *Matthiola incana*, for instance, the 7 haploid chromosomes are roughly equal in length, whereas in *M. odoratissima* one of the 6 haploid chromosomes is about twice the length of the remainder» (CATCHESIDE, 1934, p. 629). Further it may be mentioned that the chromosome number 9 of *Fritillaria ruthenica* may be derived in the same manner from the primary basic number 12 of the genus. In the case of certain *Nicotiana* species with $n = 9$ chromosomes the same mode of derivation from the general haploid number 12 of the genus has been assumed (DARLINGTON, 1937).

In some cases it is not clear whether the chromosome have undergone a fusion or the opposite process, fragmentation. In *Vicia Cracca*, for instance, SWESCHNIKOWA (1928, p. 1415) has observed three 2n chromosome numbers, viz. 12, 14 and 28. She supposes the number 12 to be primary, from which the form with 14 chromosomes could have arisen by one long V-shaped chromosome being broken and giving rise to two shorter chromosomes. DARLINGTON (l. c., p. 78) also regards this to be a case of fragmentation. BRUUN (1932, p. 162), however, points out: »As the number 7 seems to be more original, chromosome fusion would be more likely». It is, indeed, a fact that the basic number 7 is dominant in the genus *Vicia* and, moreover, is the only one present in the related genera (*Lens*, *Pisum*, *Lathyrus*; DARLINGTON and JANAKI

AMMAL, 1945, pp. 157—159). Naturally it is not justifiable to draw from these facts alone the conclusion that in *Vicia Cracca*, too, 7 would be the primary and 6 the secondary basic number. The phylogeny of the species and of the whole series of species to which *V. Cracca* belongs must be taken into consideration; in the closely related species *V. tenuifolia* only the number 2n = 24 is known.

At what stage in the evolution of the subsection *Serpylla* of the genus *Thymus* the basic number 12 (6) arose will become clear only after a thorough cytological study of the genus. Especially interesting will be an opportunity to study the most primitive representatives of the *Eu-Serpyllum* group mentioned above as well as of the Siberian forms with which ssp. *tanaënsis* is evidently connected. For the present we must be content to suppose that the original basic number in the *Serpylla* subsection is 14 (7) and that the basic number 12 found in *Th. Serpyllum* may be secondarily derived from it.

In the family Labiatae the basic number 7 is rare. In addition to *Thymus* it has been found only in two genera, *Salvia* and *Dracocephalum* (DARLINGTON and JANAKI AMMAL, l. c.). The basic number 6, on the other hand, is fairly common.

The chromosome number 2n = 54 found in *Th. Drucei* is evidently aneuploid, if it does not represent a third basic number 9, which may be proved by studies at the meiosis. For the present, however, the most adequate explanation seems to be that the species is a tetraploid, belonging to the 14-series, from which one chromosome pair would be lacking (4x — 2). This explanation, which implies that *Th. Drucei* is not directly connected with the *Th. Serpyllum* series with the basic number 12 but belongs to a parallel evolutionary series, seems to be in good agreement with the distribution of the species, with the morphological relationships, and with certain other data.

Th. Drucei is in many respects a highly specialized and without doubt a rather old species. No intermediates connect it with *Th. Serpyllum*. Also in Britain, where both the species grow in the same areas, they are easily distinguishable. It is true that in certain *Th. Serpyllum* forms some features are found which are characteristic of *Th. Drucei* (JALAS, 1947 b, pp. 48—49), but here it is a question of individual characteristics which may be regarded as convergent phenomena brought about by special climatical or other circumstances and which do not imply any genetical connection between these forms and *Th. Drucei*. Instead, we have certain forms regarded as belonging to the group *Goniotrichi* which are morphologically very close to *Th. Drucei*.

I have already earlier (JALAS, 1947 a, p. 92) pointed out that the distribution of *Th. Drucei* »is impossible to explain except on the assumption that this plant has survived at least the last ice-age on several part-areas of its present distribution», at least in Norway, Iceland and Greenland. The same conclusion has also been reached by other authors (STERNER, 1944, p. 220; DAHL, 1946, p. 237). The survival of the species and its wide distribution already during the ice-ages are the best proofs of its high age. Except in Britain the species occurs exclusively in areas where *Th. Serpyllum* is lacking, and it has clear connections to Mediterranean countries, to northern Spain. I have examined forms from there which, if not directly belonging to *Th. Drucei*, are at least very closely related to it. As we remember, the most primitive *Eu-Serpyllum* forms grow in the areas around the western Mediterranean. It must, however, be remembered that in the mountains of the Pyrenees there also occur, widely distributed, certain relatively primitive forms, included in the group *Goniotrichi*, some of which seem to be related to *Th. Drucei*. Of these may be mentioned *Th. froelichianus* OPIZ (RONNIGER, 1927 a, p. 239) and especially a variety of it, *carniolicus* BORB., an Atlantic form, which has also been found in Britain (RONNIGER, 1927 b, p. 168).

On the basis of the data presented above I am drawing the conclusion, which so far is not proved, that *Th. Drucei* originates in the Pyrenees, where it was speciated at an early period from some common ancestor of the groups *Goniotrichi* and *Eu-Serpyllum*, or from the *Eu-Serpyllum* group, before the development of the basic number 12. Thereafter it developed parallel with *Th. Serpyllum* though — already then owing to its chromosome number — quite separately from it.

My pleasant duty is in this connection to thank Professor Dr. H. FEDERLEY and Dr. E. SUOMALAINEN for their help and support during my work, and Dr. A. VAARAMA for valuable practical advice. Further I wish to express my sincere thanks to all the persons who have helped me in the collecting of material. Especially I wish to mention Professor Dr. J. A. NANNFELDT, who kindly placed at my disposal the botanical material which he has collected for the Botanical Garden of the Uppsala University. My thanks are also due to Dr. A. LÖVE (Reykjavik) and to Mr. G. BRODAL (Trondheim). I acknowledge with gratitude the kindness of Suomalainen Eläin- ja Kasvitieteellinen Seura Vanamo in supporting this study with a scholarship.

Abbreviations. — Ik, Carelian Isthmus (Isthmus karelicus). — Ka, South Carelia (Karelia australis). — Ks, Kuusamo. — Li, Inari Lapland (Lapponia inarensis). — N, Uusimaa (Nylandia). — St, Satakunta. — Ta, South Häme (Tavastia australis).

SUMMARY.

(1) The chromosome numbers of 12 *Thymus* species and subspecies, including all Fennoscandian species, have been determined. The observed numbers (2n) are 24, 28, 30 and 54.

(2) The different forms of four species (*Th. vulgaris*, *Th. pulegioides*, *Th. Drucei* and *Th. Serpyllum*) have been studied. No intraspecific variation of the chromosome number has, however, been found.

(3) *Th. Drucei* RONN., em. JALAS, earlier regarded as a subspecies of *Th. Serpyllum*, has now on the basis of its chromosome number been advanced to the rank of an independent species.

(4) It has been shown to be necessary also to pay attention to the chromosome morphology of the genus.

(5) It is regarded as probable that in respect of the grouping of its chromosome numbers the genus is most conveniently included in the »*Primula* type», in which larger natural taxonomic groups than species generally display a single basic number.

(6) The chromosome number $2n = 30$ found in the subsections *Vulgares* and *Piperellae* is absent in the subsection *Serpylla*.

(7) On the basis of chromosome morphological and phylogenetic data it is assumed that the chromosome number $2n = 24$ observed in *Th. Serpyllum* is secondary, being derived through fusion of chromosomes from the primary basic number 14.

(8) The origin of *Th. Drucei* and its relation to *Th. Serpyllum* are discussed. It has been shown that *Th. Drucei* is an old species which for the present has no connection with *Th. Serpyllum* but is more closely related to the *Goniotrichi* group in regard to its chromosome number.

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CYTOLOGICAL STUDIES OF EXTRA FRAGMENT CHROMOSOMES IN RYE

V. A NEW FRAGMENT TYPE ARISEN BY DELETION

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OF the accessory chromosomes sometimes occurring in rye (cf. MÜNTZING, 1943, 1944, 1945, 1946, 1948; HÅKANSSON, 1948) the normal type is the so-called standard fragment which is characterized by one short, headlike arm, the other arm being about five times as long. This standard fragment is not quite stable and may produce new fragment types by misdivision of the centromere. These new types correspond to twice the short arm or twice the long arm respectively. In 1945 another fragment type was observed, which evidently has not arisen by misdivision but by deletion of a segment from the long arm of the standard fragment. In the following it will be denoted d. f. (= deleted fragment).

I. THE ORIGINAL PLANT.

The new fragment type was first observed in Plant 1945—250—6. Root tips of this individual were found to contain one typical standard fragment and one smaller chromosome having the same typical short arm as the standard fragment, but the other arm much shorter than in the standard fragment (Fig. 1). At first metaphase of meiosis the same size difference was clearly seen, the two fragments generally occurring as univalents. Fig. 2 shows the typical appearance of I—M groups in this plant, and in Fig. 3 the unequal pairs of fragments from five other first metaphases are represented. Only in quite exceptional cases (much less than 1 per cent) were the fragments observed to be paired or to have been paired. Fig. 4 shows the five cases observed, and in Fig. 5 the small bivalent in the middle of Fig. 4 is shown in its natural position. As usual there was no indication at all of a pairing between fragments and normal chromosomes.

At first anaphase one or both of the fragments may pass to the poles undivided, but in most cases division occurs (Fig. 6). In five other

cells at the same stage the dividing new fragment type was drawn separately (Fig. 7). As is evident from these figures the fragments are

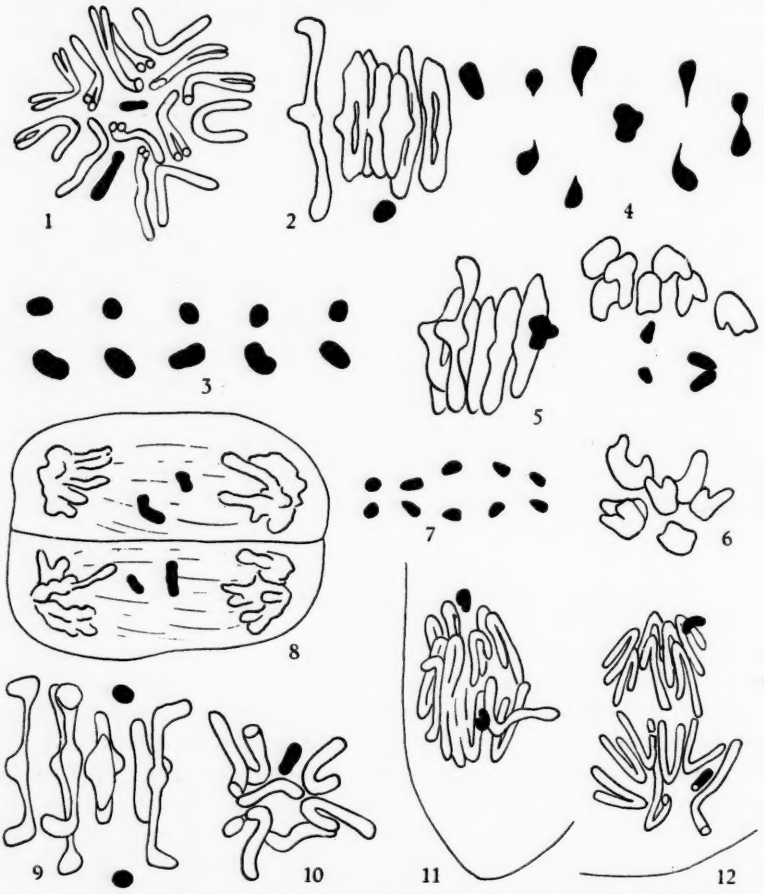

Fig. 1, somatic metaphase showing one deleted fragment and one standard fragment in addition to the 14 normal chromosomes. — Figs. 2—8, meiosis in a plant with $2n = 14 + 1$ s. f. $+ 1$ d. f. — Fig. 2, I—M with unpaired fragments. — Fig. 3, 5 pairs of fragments (separately drawn). — Fig. 4, pairing between s. f. and d. f. (from 5 different p.m.c.'s). — Fig. 5, I—M with paired fragments. — Fig. 6, I—A, division of d. f. and s. f. — Fig. 7, I—A, the d. f. has just divided (from 5 different p.m.c.'s). — Fig. 8, II—T, in each of the two corresponding anaphases lagging of s. f. and d. f. — Fig. 9, I—M in a plant with $2n = 14 + 2$ d. f. — Figs. 10—12, first pollen mitosis in plants with $2n = 14 + 1$ d. f. — Fig. 10, metaphase. — Figs. 11—12, anaphase showing normal division of the deleted fragment. — × 2400.

generally somewhat acuminate, the narrow end certainly representing the shorter arm. Owing to the division at first anaphase lagging is frequent at second anaphase. The typical case is represented by Fig. 8, showing one standard fragment and one smaller fragment lagging in each of the two corresponding anaphases.

Plant 1945—250—6, in which the new fragment type was first observed, was a daughter of Plant 1944—250—17 having $2n = 14 + 1$ standard fragment. This plant was a member of a crossing group of 7 plants, all having $2n = 14 + 1$ s. f. Owing to meiotic elimination most of the offspring of this group were free from B-chromosomes. Thus, 9 sister plants of 1945—250—6 all had $2n = 14$.

II. TRANSMISSION OF THE DELETED FRAGMENT.

Plant 1945—250—6 flowered in a greenhouse surrounded by plants having $2n = 14$. It was also pollinated with pollen from a plant having $2n = 14 + 1$ s. f. The chromosome numbers of the entire offspring were determined, the following result being obtained:

Chromosome constitution in offspring	A. 1945—250—6 × 229—6 [(14 + 1 s. f. + 1 d. f.) × (14 + 1 s. f.)]	B. 1945—250—6, open pollination (spontaneous crosses with plants having 2n = 14)
$2n = 14$	10	24
$2n = 14 + 1$ s. f.	—	1
$2n = 14 + 1$ d. f.	7	7
$2n = 14 + 2$ s. f.	2	3
$2n = 14 + 2$ s. f. $+ 1$ d. f.	1	—
Total:	20	35

In both categories (A and B) plants without B-chromosomes represent the largest class, as may, indeed, be expected. In category A the male parent will only carry standard fragments in about 7 per cent of the gametes (MÜNTZING, 1945, p. 459) and in category B the male parents were quite free from B-chromosomes. A total of 7 plants were found to carry standard fragments and of these 6 individuals had them in double number, 1 plant carrying a single standard fragment. This is quite in accordance with the non-disjunction of the standard fragment which almost regularly occurs at the first pollen mitosis and at the first division in the embryo-sac. A total of 15 plants carried the new fragment type, but in every case it was single, thus showing that non-disjunction had not occurred in this chromsome. This differential

behaviour between s. f and d. f. was further studied in the next generation. A total of 16 progenies were raised, 4 of which represented the intercross (14 + 1 d. f.) × (14 + 1 d. f.), 8 the cross (14 + 1 d. f.) × × 14, and 4 the reciprocal combination 14 × (14 + 1 d. f.). As the progenies within each category showed a similar chromosomal variation, it is sufficient to give only the total values of the three categories, the following result being obtained:

Chromosome constitution in offspring	A. (14 + 1 d. f.) × × (14 + 1 d. f.) (crossing group)	B. (14 + 1 d. f.) × × 14	C. 14 × (14 + 1 d. f.)
2n = 14	51	92	68
2n = 14 + 1 d. f.	10	3	9
2n = 14 + 2 d. f.	1	1	—
Total:	62	96	77

In the crosses (14 + 1 d. f.) × 14 and reciprocally 13 plants with fragments among a total of 173 individuals correspond to an average of 7,5 per cent gametes with one d. f. This value is quite similar to the value previously obtained for plants with a single standard fragment. Combining the male and female gametes which in 7,5 per cent of the cases contain a d. f. the resulting progeny should consist of plants having 0, 1 and 2 deleted fragments in the proportion 85,56 : 13,88 : 0,56. According to the table, the proportion actually observed was 82,3 : 16,1 : 1,6, thus showing very good accordance between observation and expectation. In the progenies of the crosses 14 × (14 + 1 d. f.) and reciprocally 13 plants carried deleted fragments. Twelve of these had only one fragment but one plant had two such fragments, this number also being observed at meiosis. In this single case non-disjunction of the fragment must have occurred. Otherwise the data clearly confirmed the evidence obtained one generation earlier, viz. that the deleted fragment has lost the ability of numerical increase by means of non-disjunction.

III. ADDITIONAL OBSERVATIONS ON MEIOSIS AND POLLEN MITOSIS.

To the cytological evidence obtained from the original mother plant (Figs. 1—8) some further data from plants in the offspring may be added. These may be grouped under the following headings:

1. MEIOSIS IN A PLANT WITH 2 d. f.

In the exceptional plant described above, which represents a case of non-disjunction of the d. f., the two fragments were found to be unpaired at first metaphase (Fig. 9). This was found to be the case in all of 53 p.m.c.'s observed at this stage. At first anaphase division of both fragments was seen in 8 cells, division of one fragment in 4 cells, in 2 cells one fragment was eliminated, and in 4 cells the fragments had passed to the poles without division. Thus, division of the fragment at I—A is quite frequent.

2. MEIOSIS IN PLANTS WITH 1 d. f.

Meiosis was studied in 10 plants having $2n = 14 + 1$ d. f. in the root tips. In eight of these plants the fragment was also regularly present at meiosis, in one plant it was entirely absent and in one plant it was present in some anthers but absent from at least two loculi. In these loculi hundreds of p.m.c.'s at I—M were observed, none of them containing the fragment, whereas other loculi visible in the same section regularly showed the fragment to be present. This chimaerical structure demonstrates that in this case the fragment had been eliminated from some tissues at a rather late stage. In the other plant, entirely lacking the fragment at meiosis, elimination had evidently occurred earlier. These data indicate a lower degree of mitotic stability in the new fragment type than in the other types of B-chromosomes so far studied in rye, standard fragments as well as iso-fragments very seldom showing any difference in number in root tips and at meiosis.

Without exception the new fragment type was found to be unpaired at I—M and had the same appearance as the unpaired fragments in the plant with $2n = 14 + 2$ d. f. (Fig. 9). In about half of the first anaphases observed the fragment was seen to divide or to be lagging. In one of the plants second anaphase was observed. In 43 p.m.c.'s the two corresponding anaphase groups were regular, showing no fragments, in 20 p.m.c.'s both corresponding anaphases contained a lagging fragment and in 2 cells a lagging fragment could only be seen in one of the anaphase groups.

3. POLLEN MITOSIS IN PLANTS WITH 1 d. f.

Though there is a rather high degree of meiotic elimination of the single fragment in plants having $2n = 14 + 1$ d. f., a few meta- and anaphases of the first pollen mitoses were observed. Fig. 10 shows the appearance of the new fragment type at metaphase of the first pollen

mitosis. Figs. 11—12, showing anaphase of the first pollen mitosis, are of special interest, as they clearly show that the fragment has divided just like the other chromosomes and does not show any tendency to non-disjunction. Figs. 11 and 12 are from different plants and just the same picture was also observed in a third plant. This behaviour at the pollen mitosis was indeed to be expected from the experimental data. Also in this material p.m.c.'s containing the fragment appeared to be slightly slower in development than those which are free from the fragment (cf. MÜNTZING, 1946).

IV. RELATIVE SIZE OF STANDARD FRAGMENT AND DELETED FRAGMENT.

In order to compare the size of s. f. and d. f. some measurements of these chromosomes in somatic plates were undertaken. The results obtained are given in Table 1. These data were gathered from the original plant (No. 11328) and from 10 different plants in the offspring, 5 containing the deleted fragment and 5 containing standard fragments. The best comparison between s. f. and d. f. is offered in the original plant ($2n = 14 + 1$ s. f. $+ 1$ d. f.), in which the two fragment types could be measured in the same somatic plates. According to the table, the average size of d. f. in this plant is 7,36 units, the corresponding value of s. f. being 13,64. Each unit corresponds to 0,24 μ. Thus, the length of the new fragment type was 54,0 per cent of the length of the original fragment. In the offspring (representing two generations) the average value of the d. f. was somewhat higher, or 8,28, but the average value of the s. f., measured in 5 plants taken at random, was also somewhat higher (13,96). This gives a percentage value of 59,3. As the short arm of the standard fragment represents about one fifth of the total length and as the new fragment type seems to have the same short arm as the s. f., it may be concluded that the origin of the d. f. type involved the loss of about half the long arm of the s. f.

The data in Table 1 show a rather marked inter-plant variation especially in the d. f. category. This was tested with an analysis of variance, the heterogeneity being significant in the d. f. as well as in the s. f. category. In both cases P is smaller than 0,001, but in the d. f. category variation between series is much more pronounced than in the other category. The reason of this differential behaviour is not clear. It might possibly imply further structural variation of the d. f. type, the s. f. being structurally constant. However, the significant inter-plant

variation in size of the standard fragment must have a special cause and this cause is certainly a variation in degree of chromosome contraction. The inter-plant variation in this respect is a rather striking phenomenon in rye and may be observed at mitosis as well as meiosis. This variable degree of contraction is also responsible for at least part of the inter-plant variation in the d. f. category. It is possible that for some reason so far unknown the deleted fragment reacts more intensively than the standard fragment to the forces influencing the degree of contraction.

TABLE 1. *Length of deleted fragment and standard fragment.*

Category	Number	Chromosome length (1 unit = 0,24 μ) 5	6	7	8	9	10	11	12	13	14	15	16	n	M
Deleted fragment	11328 (mother pl.)			6	12	3								21	7,36
	13033					6	3	1						10	9,00
	13056	2	6	2										10	6,50
	15399					4	5	1						10	9,20
	15413				1	6	3							10	8,70
	15573				5	5								10	8,00
	13033—15573, total	2	6	8	21	11	2							50	8,28
Standard fragment	11328 (mother pl.)								6	9	3	3		21	13,64
	13034								4	5	1			10	13,20
	13062									6	4			10	13,90
	15641								3	2	3	2		10	13,90
	15642								2	3	3	2		10	14,00
	15643									1	5	4		10	14,20
	13034—15643, total								9	17	16	8		50	13,96

V. CONCLUSIONS.

The data presented above demonstrate that the new fragment type observed in rye is derived from the so-called standard fragment (of the variety Östgöta Gråråg) and has arisen by deletion of about half the long arm of the standard fragment. Chromosome counts in two generations among the offspring of the original plant demonstrate that the deleted fragment has almost entirely lost that ability of non-disjunction at the first postmeiotic mitosis which is a characteristic of the standard fragment. This loss affects the male as well as the female side, as was shown by the results of the crosses $14 \times (14 + 1$ d. f.) and reciprocally. Direct observations of the pollen mitosis revealed, indeed, that the deleted fragment divides regularly like the normal chromosomes.

Non-disjunction of the standard fragment (as well as the large

iso-fragment) was assumed to be due to a special, probably heterochromatic segment of the long arm not far from the centromere (MÜNTZING, 1946). The deletion giving rise to the new fragment type evidently included this special segment. The fact that the new fragment type has lost a segment of the long arm and the ability of non-disjunction simultaneously strongly supports the previous conclusion, based on other evidence, that non-disjunction in the standard fragment is caused by a special segment in the long arm.

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INHERITANCE OF ANTIGENIC DIFFERENCES IN COLLYBIA VELUTIPES

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MANY antigenic differences have been found to be gene determined. The genera mainly investigated in this respect are man, cattle, dove, pigeon, rabbit, duck, and fowl. Among lesser studied are horse, mouse, and dog. For elucidation of various aspects of the problem and details reference may be made to reviews by IRWIN (1946, 1947) and WIENER (1946).

For the present genetical investigation of antigenic characters, the fungus *Collybia velutipes* (Agaricaceae) was chosen for the reasons that it is easily cultivated on malt agar, gives a good crop of mycelium and develops fruiting bodies on agar under favourable conditions. The chances of desired cross fertilization are also satisfying, sex depending on two pairs of genes. Further, the genetical analysis is simpler where the haplophase, as here, can be used in the genetical and serological tests.

The life-cycle of *Collybia* consists in short of a haplophase of basidiospores which give rise to the haploid mycelia. The diplophase arises when mycelia from spores with different genes for sex are combined, the cells of the resulting mycelium and fruiting body having two nuclei. Fusion of the two nuclei occurs in the basidium and is immediately followed by reduction division. Each basidiospore receives one nucleus and is haploid.

The original material was obtained from a natural habitat in the neighbourhood of Stockholm.

From the collected fruiting bodies forty-nine separate spores were isolated and the mycelia propagated. Injections of rabbits with mycelium showed that antibody-formation against this fungus occurred and an antiserum was prepared against one of the mycelia, No. 2, from the same spores. This serum was used in complement-fixation tests with each one of the other forty-eight mycelia as antigen for the purpose of finding, if possible, antigenic and genetic differences in haploid individuals for further analysis. The tests showed that six mycelia, used as antigen, had nothing in common with the the antiserum from No. 2,

haemolysis occurring, whereas the other forty-two mycelia gave no haemolysis.

The six individuals which thus were shown to differ from No. 2 were respectively crossed with No. 2. From one of the crosses, No. 2 × No. 130, fruiting bodies were collected and spores were isolated, which were given numbers 301—419.

After having obtained antiserum against the parent individuals, Nos. 2 and 130, the descendants, 103 individuals of Nos. 301—419, were used in complement-fixation tests with the parents. It appears from the results that all individuals in this family, except two (Nos. 379 and 383), had some factor in common with both parents. Nos. 379 and 383 had one factor or more in common with one of the parents and none with the other. The parents, Nos. 2 and 130, would hence differ in several factors, and the segregation bears out the serological test which showed that Nos. 2 and 130 had nothing in common.

For further analysis, it was necessary to find and, if possible, identify differences between the individuals in this family, 301—419. Antiserum was therefore made against some of these, Nos. 305—416, including No. 383, and the sister individuals, Nos. 301—419, were tested with these antisera. The complement-fixation tests show the number of individuals respectively with some factor in common (+) and none (0) in common with the sister individuals:

For antiserum No.	Individuals with +	0
333	103	1
383	50	32
309	96	1
315	100	2
323	91	5
332	95	2
305	97	4
316	79	22
364	100	4
410	101	3
398	86	1
416	84	2

Though the numbers in the 0 class are small one might infer, for instance, that Nos. 323, 305, 364 and 410 agree with an expectation based on four dominant factors (common to antiserum and a pro-

portion of individuals), χ^2 of the ratio for No. 323 being 0,1778, for 305 0,9036, for 364 1,0256 and for 410 2,0102. An agreement with expectation on two factors occurs in No. 316, χ^2 being 0,5577. For 383 neither the assumption of one or two factors is realised, the numbers probably being incorrect due to uncertainty in scoring some of the weaker individuals.

It is apparent that a gametic ratio here of, for instance, 3 : 1 for No. 316 must indicate that this individual only has two dominant factors in common with some of the sister individuals, No. 316 being, for example, *AbCdef* . . . and the sister individuals being gametes from a hybrid heterozygous for *AC* and more factors. As the ratio is the result of trial between one individual of a family with sister individuals in the same family, the 3 : 1 ratio cannot be due to No. 316 having more than two dominant factors and the sister individuals being gametes from a hybrid heterozygous for only two factors. A 3 : 1 ratio may of course arise from trial of an antiserum with two factors (e. g., *AbCdef* . . .) with any family with the corresponding two dominant factors together with any number of further factors (e. g., from a hybrid *AaCcBbDdEeFfgg* . . .). The same applies to the other ratios.

The ratios above give no information about the number of factors occurring in family 301—419. The individuals above, 333—416, in this family can, however, be grouped according to behaviour when tried only *i. se*, the groups occurring being:

Antigen No.	Antiserum 305, 333, 309, 315, 332	383	323	316, 398, 416	410, 364
383	0		0	+	+
309	+	0	+	+	+
315	+	0	+	+	+
323	+	0		+	0
332	+	0	+	+	+
305	+	0	+	+	+
316	+	+	+	+	+
364	+	+	0	+	+
410	+	+	0	+	+
398	+	+	+	+	+
416	+	+	+	+	+

While three numbers (316, 398, 416) have some factor in common with all the others, five numbers (305, 333, 315, 332, 309) differ only from 383. Two numbers (410, 364) differ only from 323, one number

(323) differs from 364, 410 and 383, one number (383) differs from 309, 315, 323, 332 and 305. As number 383 has two factors (see below), it may be designated *AB*. 316 having also two factors and something in common both with 383 and 323 may be termed *AC*. 323 has four factors of which none occurs in 383 but one in 316. The factor common to 316 and 323 would be *C* and 323 would be *abC* with three more factors, e. g., *DEF*. 410 and 364 having four factors, none of which is common with 323, must be *cdef*, and as these numbers give + with 383 and 316, they must have the factor *A*. Moreover, three further factors are required, e. g., *G* and *H* and possibly *B*. Number 305 has four factors and since it has nothing in common with 383, but something in common with 316, it would be *abC* with the addition of three more factors, one or two (beside *C*) of which may be common with 323. Since 305 gives + with 410 and 364 the factors *C* and/or *H* may be present in 305.

About the remaining individuals in the 305 group, i. e., 333, 309, 315 and 332, which seem to have more factors than 305, no inference can be made except that they are *abC* and do not belong to the other groups. The same applies to Nos. 398 and 416 in the 316 group, which probably are *AC* with one or more factors.

These inferences may indicate the presence of at least eight factors in this family and the hybrid 2 × 130 must have been heterozygous for all; if homozygous for any factor, this would have made an analysis in this way impossible.

For further analysis of No. 383, which number seemed to be factorially the most simple, it was crossed with No. 323, having probably four factors (*abCDEF*). The two numbers were also chosen as they have nothing in common. Spores, Nos. 500—602, from this cross were bred and used in complement-fixation tests as antigen with antisera against the two parents, 383 and 323. Of the 89 individuals in this family tested with the 383 antiserum, 70 gave + and 19 gave 0, showing that 383 actually had two factors (*ABcdef*) ($\chi^2 = 0{,}58803$). Tested with antiserum 323 there were 82 with + and 7 with 0 ($\chi^2 = 0{,}39625$), which is, as when the number was tested above, a close fit with expectation on four factors for 323. Further, tested with 316 there were 72 individuals with + and 17 with 0, which probably bears out the assumed two factors for 316.

The individuals in family 500—602, derived from 383 × 323, can be grouped according to behaviour when tried with antisera 323, 383 and 316, as seen in Table 1.

The groups correspond to the classes of individuals which may be

TABLE 1.

Group of individuals	Antisera			Genetic constitution of the group		Obtained individuals	Expected
	383 *ABcdef*	323 *abCDEF*	316 *AbCdef*				
I	+	+	0	*aBc*	*D* or *E* or *F* (single, two or all factors)	6	7/64
II	+	0	0	*aBcdef*	—	2	1/64
III	+	0	+	*Acdef*	with *B* or without	4	2/64
IV	0	+	0	*abc*	*D* or *E* or *F* (single, two or all factors)	8	7/64
V	0	+	+	*abC*	*D* or *E* or *F* (single, two or all factors)	10	8/64
VI	0	0	0	*abcdef*	—	1	1/64
VII	+	+	+	the remainder		58	38/64

expected to occur in the family and which may be identified with these three antisera. Using only these three antisera, the *D*, *E* and *F* factors in groups I, IV and V could of course not be separately identified, whereas their complete absence was demonstrated in groups II, III and VI.

Though all expected groups are realised and the individuals in each group seem to occur in reasonable proportions, the numbers in groups II, III and VI are small (below 5). As these groups contain *cdef*, they may be pooled, in which case the groups and number of individuals would be

	I	II, III, VI	IV	V	VII
obtained	6	7	8	10	58
expected	7/64	4/64	7/64	8/64	38/64
	9,734375	5,56250	9,734375	11,125	52,343750

The numbers obtained agree with expectation, the genes probably being independent.

DISCUSSION.

Since it is evident that antigens recognizable by immunological methods are definite chemical substances, studies on the causation and

mode of inheritance of antigenic characters might, in combination with a chemical analysis throw some light on the constitution and relations between the genes and their effects. Various aspects of the problem have recently been treated especially by MULLLER (1947), further by IRWIN (1947), LANDSTEINER (1946), WIENER (1946), BOYD (1945), and BOYDEN (1942).

In spite of the investigation of many genera comparatively little is known about the genetics of antigenic differences, and even in the most thoroughly studied species only a few of probably many antigens have so far been investigated or shown to be gene determined. Still less is known about the interaction of the responsible genes and of their mode of action.

That many antigenic differences may exist inside the species appears from the work of TODD (1913) on cattle and (1931) on chickens, from that of LEVINE and LANDSTEINER (1931) on rabbits and especially from the investigations of cattle by FERGUSON, STORMONT and IRWIN (1941, 1942). About forty blood cell antigens have thus been found in individuals of cattle (STORMONT, IRWIN and OWEN, 1945 and unpublished data mainly by CLYDE, STORMONT and OWEN; according to IRWIN, 1947), the data indicating that the responsible genes are located in ten of the presumably thirty pairs of chromosomes. In man four of the twenty-four chromosomes would be marked with genes for blood antigens if the genes *Rh* and *P* are independent. That only a few alleles need to be taken into account to explain the various differences in chicken blood was shown by WIENER (1946) in an analysis of the data of TODD (1931). Further, in the extensive work on the common and species-specific antigens of doves IRWIN and CUMLEY (1947) come, for instance, to the conclusion that in *Streptopelia senegalensis* at least thirteen or fourteen of the presumably thirty chromosomes have one or more genes for cellular and serum antigens which are specific to the species and differentiate it from *St. risoria*. Similarly *St. chinensis* differs from *St. risoria* in having one or more genes for blood cell antigen in ten chromosomes and a minimum of three chromosomes with genes for species-specific serum antigens (IRWIN, 1947). It would thus seem that only a part of or up to about half of the chromosomes of the respective species have genes for specific antigens and that the antigens are not produced by genes, situated on many chromosomes, with multiple effects.

Besides single alleles series of alleles and/or possibly linked genes for antigen differences occur. Further work may show if the *Rh* sub-

types in man depend on a series of alleles as upheld by WIENER and co-workers (1946) or on linked genes as proposed by FISHER and RACE (1946). The same problem exists also in cattle (STORMONT et al., 1945) and ducks (MC GIBBON, 1945, 1947). The experimental results in cattle and ducks show that on an interpretation of a series of allelic genes, individual alleles in the series may control the production of more than one antigen and in man the Rh_1 produces the same effect as *Rh* and *Rh'* together and Rh_2 the same effect as genes *Rh* and *Rh"* (WIENER, 1943). Much interest attaches also to the further elucidation of the situation in the Columbidae, where, for instance, each of the cellular components distinguishing *Streptopelia senegalensis* from *St. risoria* behaves as if it was produced by the joint action of genes on a particular Segenal chromosome (IRWIN and CUMLEY, 1947).

Although most cellular antigens seem to be produced by a specific gene, complementary genes were reported by THOMSEN (1936) for chicken. There is further a »hybrid substance» in the hybrids between *Streptopelia chinensis* and *St. risoria* and between the latter and *Columba livia* (IRWIN, 1932; IRWIN and COLE, 1936), as also in the hybrids between duck species (MC GIBBON, 1944). This substance in the first mentioned hybrids has been divided into three parts (IRWIN and CUMLEY, 1945).

The only cases of linkage between a gene for an antigen and a gene for another character so far found are in rabbits (SAWIN et al., 1944) where an antigen is linked with brachydactyly, and in ducks (MC GIBBON, 1946), where linkage with sex is reported. There seems also to be an association between *Rh* and feeblemindedness (SNYDER et al., 1945).

In lower organisms, though specificities between antigens and antibodies are well known, the material is mostly not suited for a genetical investigation along usual lines. Organisms such as *Paramecium* are, however, open to genetic investigation, the inheritance of antigens as well as other characters having been studied by SONNEBORN (1947) and others. While there are no interspecific cross reactions in the *aurelia* group of *Paramecium*, such are found between various stocks inside the species (BERNHEIMER and HARRISON, 1940, 1941) and could be distributed into groups. The principal antigen differentiating stock P, in variety I of *P. aurelia*, is determined by a single dominant gene (SONNEBORN, 1943). The case also demonstrates »cytoplasmic lag» or »maternal effect» since recessive clones, derived from the hybrid when this was selfed or crossed with the recessive, still showed the dominant character

during four to eight fissions, thereafter disappearing. Also, when the dominant gene is introduced into the recessives the antigen cannot be detected in the heterozygotes for several fissions, after which there is a gradual increase of the antigen until it reaches normal level.

The present investigation shows the possibility of analysis of antigenic components in fungi, a class of organisms which might be amenable to a chemical analysis of the antigens. No information was obtained about the number of antigenic components occurring. Those antigens which were examined may possibly be referred to about eight chromosomes.

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SUMMARY.

(1) A family obtained from crossing two mycelia which had no antigen in common showed segregation of genes for antigen, as determined by complement-fixations tests.

(2) When the individuals in the family were tested *i. se.* the ratios indicated that some of the individuals had two genes for antigen, others had four, and still others possessed more genes for antigen.

(3) From comparison of these individuals the inference is drawn of at least eight genes for different antigenic components in the family.

(4) A cross between an individual with two genes for antigen and another with four different genes for antigen bore out the constitution suggested of the respective *P* individuals in the cross.

(5) The individuals in this family, when tested with antisera against the parents and another individual of known constitution, can be grouped according to behaviour, the groups and number of individuals in the respective groups probably indicating an independent assortment of the genes for the antigenic components.

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INDUCTION OF CHROMOSOME FRAGMENTATION BY PHENOLS

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I. GENERAL OBSERVATIONS.

SINCE our recent discovery (LEVAN and TJIO, 1948) that phenols have a fragmenting effect on *Allium Cepa* chromosomes we have tested some forty phenols, phenol derivatives and related compounds on their fragmenting activity. Although the majority of them exhibited some activity, most of them were found to be but very little active. In many cases only a systematic search through many slides gave a positive result.

This may be one reason for the non-detection of this reaction earlier in spite of its wide distribution within this type of substances. Another reason is that the reaction may be well-defined and distinct only in favourable cytological materials. Thus, PARMENTIER and DUSTIN (1948) evidently deal with the same reaction in the mouse after hydroquinone treatment. They mention, in fact, a chromosome fragmentation as one among four alternative explanations of their findings, but they consider it as more likely that some change in the centromeres induced by the treatment is responsible for the characteristic cytological picture at hand. »On each side of the metaphase plate little groups of chromosomes migrate towards the poles. These chromosomes are usually the smallest ones», and later: »An intrinsic cause of the abnormal chromosome migration appears to be the most probable. It is suggested that an impeded division of the centromeres of the longest chromosomes gives the best explanation of the peculiar mitotic figures» (l. c., p. 527). In the more favourable material of *Allium* it is easily seen that the »smallest» chromosomes migrating to the poles are the 16 centromeres together with so much of their chromosome arms as has been left, while the remainder of the chromosome matter lags at the equator region as free fragments.

Our standard method of testing the substances on their fragmenting activity is the same as LEVAN and collaborators have employed since 1938 for analysing the c-mitotic activity of chemical substances. A

dilution series of the substance to be tested is prepared, consisting of 13—19 concentrations covering the region 0,1—0,000·01 or, in some cases, 1—0,000·000·1 mol/l. Small *Allium Cepa* bulbs of the Zittauer variety, which have been grown on tap-water until their roots are about 1 cm in length, are then moved over to these solutions. The roots are observed macroscopically for demonstration of toxicity; in the highest concentrations they often lose their turgor. After one day in the solutions the c-tumours (LEVAN, 1938) begin to appear. Cytological fixations are made on two occasions during the treatment, viz. after an exposure of 4 and 24 hours. These fixations supply material for the analysis of c-mitosis and chromosome fragmentation.

In the present paper few details are given of the morphology of these reactions, the aim being to record the occurrence of fragmenting activity among the chemicals tested. However, some general cytological features characteristic of these treatments will be briefly described in this first chapter. In the strongest concentrations toxicity is usually high, and the tissues are instantly killed by the treatment. Consequently, in this first concentration zone (below called zone 1), no c-mitoses and no anaphase bridges are found. A certain minimum of vital processes is necessary if these reactions are to be manifested. The dying off of the tissue is often very characteristic in its cytological type. Various phenomena connected with the so-called agglutination of the chromatic material predominate in zone 1, but may also reach down far below this lethal zone. The matrix of the chromosomes is liquefied and has a tendency to flow out along the borders of plasma vacuoles or to be lumped together into one or more homogeneous chromosome clusters. These phenomena are often referred to as pycnosis, and according to the current cyto-chemical view they are due to depolymerization of nucleic acids.

While the pycnotic type of changes is of limited chromosome-morphological interest, other changes occurring in consequence of the liquefaction of the chromatic material are of greater interest. Often the stainable substance is gently washed out from the chromosomes, revealing a delicate internal structure. Although the chromosomes are dead, these structures may be of significance for the interpretation of vital structures. Some features of the chromosome morphology may be approachable only after the chromosomes have been killed under well-defined conditions. Thus, in some of our treatments the subdivision of the chromosomes into half-chromatids is very clearly seen. Another typical reaction which only appears after quite particular treatments

is the differential staining of the heterochromatin during contraction stages. Especially the proximal or prochromosomic heterochromatin, but in some cases also the distal, end heterochromatin retains the stain stubbornly. Such stainings as were described after killing the roots in mercuric nitrate (LEVAN, 1945, 1946) are often found in the present treatments within zone 1. Cresols, for instance, show these differential prochromosomic stainings regularly and through the entire meristem, while mercuric nitrate only had this effect in the superficial layers. In extreme effects of these types the entire metaphase chromosomes with the exception of the centromeric region may be dissolved, »free» prochromosomes remaining on the equatorial plate. This effect described by STEINEGGER and LEVAN (1947 b) for chloroform is found, for instance, in naphthol treatments.

Often a more irregular mottling of the chromosomes is brought about, stainless and stained regions alternating. All types occur from chromosomes with only a few unstained spots and such with a more equal mottling of the entire chromosome complement to those which are without colour except for one or two deeply stained spots. The transition from stained to unstained area is sharp but the distribution of each area may be quite irregular, it may involve the whole chromosome breadth or only a section of it. Even if the stainless substance is quite transparent it can be seen that the chromosome outline is undamaged all around the chromosome. On staining with Feulgen the stainless areas also acquire a red colour, though often paler than the rest of the chromosome. This effect is hereafter referred to as »mottling».

Below zone 1 there comes a concentration region where c-mitosis is prevailing (zone 2). This reaction may be complete in one or two concentrations, above and below which there occur mixed c-mitoses and normal mitoses. In the former case (higher concentrations) the presence of normal divisions is due to lack of viability, which keeps normal divisions preserved from before the treatment to the moment of fixation. Only a few mitoses are viable enough to assume the c-type. In the latter case (lower concentrations) the presence of normal mitoses depends on the proximity of the lower threshold of c-mitosis. The concentration is not high enough to give c-mitoses in all dividing cells. Zone 1 may partly overlap zone 2, in which case we may get c-mitoses with mottled chromosomes.

The next concentration region, or zone 3, is characterized by normal mitoses, and it is here that chromosome fragmentations are

found. It is only in exceptional cases that fragments are found in c-mitoses. In concentrations giving mixed c-mitoses and normal mitoses (i. e. the lower part of zone 2) fragments may be found but almost exclusively within the normal mitoses. This is not only due to the greater difficulty of identifying fragments within a c-mitosis, where no chromosome movements occur, for clear fragments have also been observed in normal metaphases (Fig. 1). It is evident that the frag-

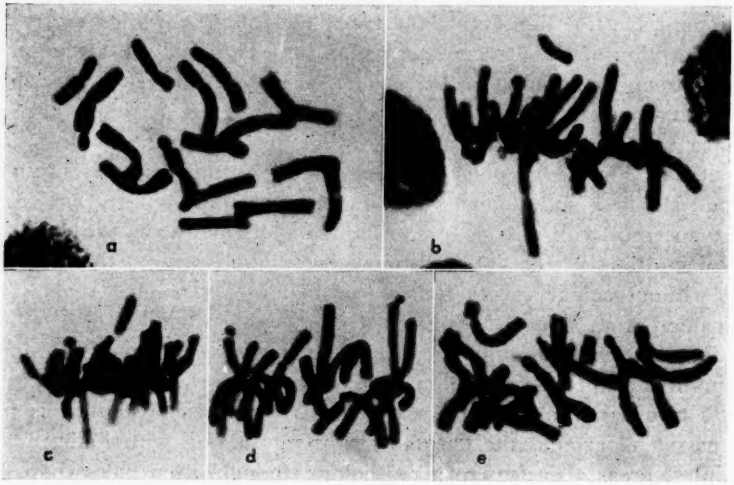

Fig. 1. *Allium Cepa*, Feulgen smears of root chromosomes. — *a*: metaphase in polar view, in two chromosomes fragmentations have occurred close to their ends; *b*—*e*: metaphases in side view; *b*: one large fragment outside the plate, in addition one attached fragment; *c*: one large attached fragment; *d*: two small attached fragments; *e*: in one chromosome both sister chromatids are attacked, one is completely severed, the other one has only developed a constriction. — × 2000.

mentation needs a certain measure of viability: the frequency curve of fragmentation in different concentrations of the same substance always shows an optimum falling both towards higher and lower concentrations. Generally speaking the c-mitotic and the fragmentation reactions exclude each other.

In order to attain to an estimation of the fragmenting activity of each concentration of a substance tested we have studied a number of anaphases and determined the number of fragments in each cell. The frequency of cells with fragments expressed as percentage of the counted cells (called fr %) gives a picture of the activity. It is evident that each

TABLE 1. *Fragmentation percentage in different roots of four treatments with 0,000·5 mol/l (concentration 8) pyrogallol.*

Slide No.	Date of fixation	Root No.	Number of cells with fragments							Mean number of fragments per cell	Fragmentation %	Total cells analysed
			0	1	2	3	4	5	>5			
40820	Nov. 11, 1947	1	13	4	6	6	4	4	—	1,9	64,9	37
		2	12	8	7	1	3	1	1	1,5	63,6	33
		3	16	8	6	6	1	6	4	2,0	66,0	47
		4	17	6	5	2	1	3	3	1,6	54,1	37
		5	5	4	5	1	3	1	1	2,0	75,0	20
		Total	63	30	29	16	12	15	9	1,8	64,8	174
42567	March 2, 1948	1	26	15	11	3	6	4	—	1,4	60,0	65
		2	31	7	3	3	—	1	—	0,6	31,1	45
		3	13	9	5	2	2	—	2	1,4	60,6	33
		4	13	11	4	2	2	1	3	1,6	63,9	36
		5	25	10	7	2	5	3	—	1,3	51,9	52
		6	24	7	3	1	1	1	1	0,8	36,8	38
		7	28	4	5	2	—	1	3	1,0	34,9	43
		8	75	30	12	7	3	6	7	1,1	46,4	140
		9	70	8	6	6	2	1	—	0,6	24,7	93
		Total	305	101	56	28	21	18	16	1,0	44,0	545
42607	March 17, 1948	1	43	15	7	—	—	—	1	0,5	34,9	66
		2	42	3	2	—	—	—	—	0,2	10,6	47
		3	44	11	4	1	4	1	3	0,9	35,3	68
		4	16	4	3	—	—	—	—	0,4	30,4	23
		5	39	15	4	1	1	—	—	0,5	35,0	60
		6	26	7	1	—	—	—	—	0,3	23,5	34
		7	28	5	2	5	1	1	1	0,9	34,9	43
		Total	238	60	23	7	6	2	5	0,6	30,2	341
42718	April 2, 1948	1	50	10	—	—	—	—	—	0,2	16,7	60
		2	60	3	2	3	—	—	—	0,2	11,8	68
		3	25	6	2	1	—	—	—	0,4	26,5	34
		4	20	1	—	—	—	—	—	0,1	4,8	21
		5	45	8	1	2	—	—	—	0,3	19,6	56
		6	40	14	8	1	1	—	—	0,6	37,5	64
		Total	240	42	13	7	1	—	—	0,3	20,8	303

determination of fr % is liable to considerable error. In order to judge the reliability of the individual fr % value we made some control counts of slides from different treatments with the same concentration of one substance and from different roots that had undergone the same treatment. As expected, we found a considerable variation. Anyhow, according to our opinion, the activity of a substance is rather well-described if at least 100 cells of each concentration within the series have been analysed. Often, however, various circumstances, as for instance lacking number of divisions, have cut short the counting at a lower number of cells. Especially after 24 hours the toxic effect is usually high even in zone 3 and constitutes a source of error. Counts after 4 hours have therefore mainly been used for judging the activity of a certain substance. The results of the longer treatments furnish only a complement to this. Only when really new characteristics have been revealed by the longer treatments have these also been taken into consideration. Usually the change in the picture from 4 hours to 24 hours is as follows. Higher concentrations of zone 3 may either give a complete lack of mitoses or a small number of mitoses with high fragmentation frequency. Lower concentrations, where toxicity is low, usually give a decreased fr % as compared with 4 hours' treatment.

As an instance of the variation in fr % Table 1 is submitted. It contains the results of fragment counts on four different occasions within the most active substance. In each slide the individual roots were recorded separately. The variation between various treatments is great, from 21 to 65 %. If only one root had been counted on each occasion it would have been possible to get as different values as 4,8 and 75,0 %. Within each fixation less variation is found, the limits being denoted by the ratio 1 : 5. We do not know what is the cause of the variation, but even small variations in the external conditions may presumably cause considerable differences in the reaction.

Since many of the compounds employed are easily oxidizable and undergo rapid change in tap-water, we considered that the conditions could be kept under better control if we used distilled water for our dilution series, although we are aware that distilled water, especially during long treatments, acts poisonously on the onion roots. We made, however, several comparative series as between tap-water and distilled water and found that the fragmentation also occurred in tap-water, though less regularly. This problem will be taken up later, especially as regards the c-tumour reaction, which is much more easily brought about in distilled water than in tap-water.

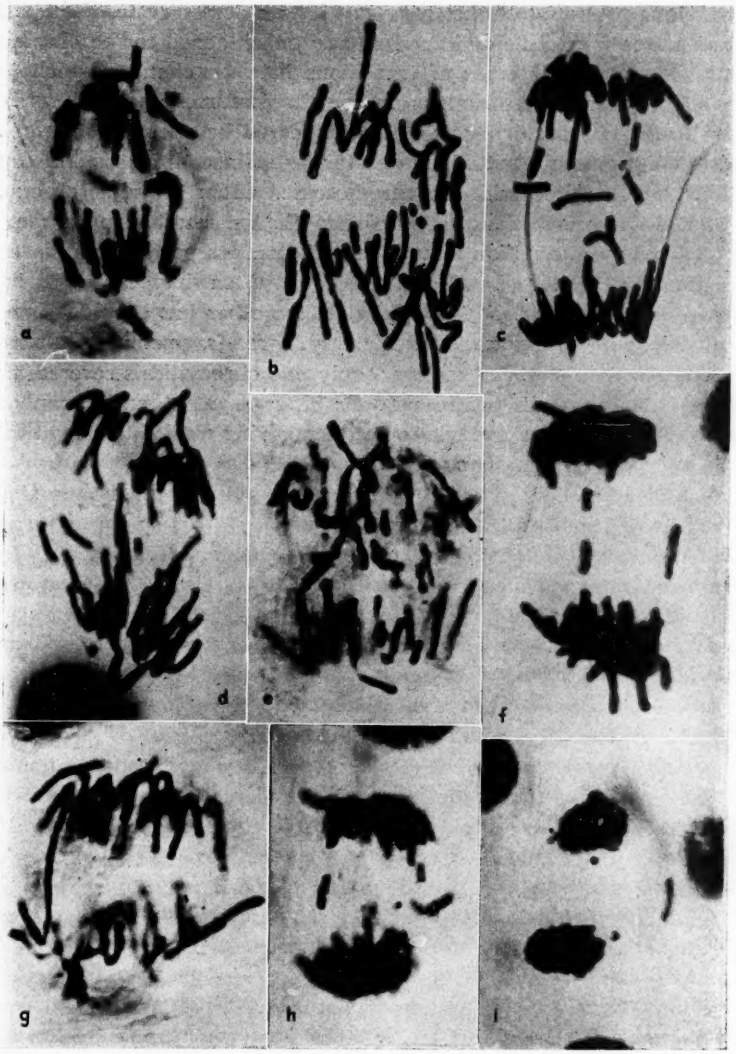

Fig. 2. *Allium Cepa*, Feulgen smears of root chromosomes (*a, d, e* sectioned roots stained in crystal violet). — *a—e, g*: anaphase, *f, h*: late anaphase, *i*: telophase. *a, d, e*: very extreme changes, many constrictions and fragments; *b*: two small fragments; *c*: 6 free and one attached fragments; *f*: 3 fragments; *g*: translocation bridge, indicative of sister reunion; *h*: several fragments; *i*: two micronuclei and one large fragment. — × 2000.

At our recording of the fragments it was necessary to distinguish between free fragments and such as are still attached to the chromosome. There are all kinds of transitions between changes which represent only small constrictions on the chromosomes and such where most of the chromosome-arm has disappeared between the fragment and the chromosome but where one faintly stained thread is still connecting the fragment with the chromosome. Often the same chromosome arm is eroded along its whole length, the result being a rough outline and an alternation of stained and stainless portions within the chromosome (Fig. 2 *a*). At metaphase it often happens that one chromatid is eroded right through while the other has only developed a constriction at the same level (Fig. 1 *e*). Free fragments are already present at metaphase. If, however, only one chromatid is severed, the fragment will remain paired with its sister segment until anaphase starts. Therefore, free metaphase fragments lying outside the equatorial plate are comparatively rare; they are double and consist of both sister chromatids (Fig. 1 *b*). Metaphase is consequently no favourable stage for counting fragments. In some slides, however, very many chromosomes were seen to be fragmented already at metaphase; in some cases we found that a fragmentation often occurred at about the same level, at a little distance from the chromosome end (Fig. 1 *d*). We dare not claim, however, that this is due to a certain chromosome region being especially susceptible to fragmentation, but we have our attention directed to this problem for future analysis.

At anaphase the distinction between free and attached fragments and merely eroded chromatids is made apparent. Free fragments remain at the equator, whereas the attached fragments are pulled by their chromosomes towards the poles. It is sometimes difficult to see the attachment thread, but the position of a certain fragment often gives an indication as to whether it is free or not. The free fragments usually orientate themselves transversally on the spindle, while the attached fragments, of course, are situated lengthwise. In our records only undoubtedly free fragments are counted, our counts therefore giving minimum frequencies of fragmentation. Since the two fragment-types accompany each other through all treatments they are considered to represent different aspects of the same reaction. Perhaps a less severe attack on the chromosome thread results in a constriction, while a somewhat harder attack gives a free fragment.

The free fragments may have any size from very small »subminutes» to entire chromosome arms. Accordingly, the centromeric

part of the chromosomes varies very much; in some cases only the smallest prochromosomic part of the anaphase chromosome is seen to proceed to the pole (Fig. 2 *d*, at 7 o'clock). Those small chromosomes are often ahead of the large chromosomes during the pole wandering. If the pull on the centromere is the same in all chromosomes, those which have lost their arms should furnish less resistance to the plasm than the undamaged chromosomes. Fig. 2 gives some instances of attached and free fragments at anaphase—telophase. While *a* contains only eroded chromosomes, *b* and *c* have several clearly free fragments. Fig. 2 *d* and *e* are more extreme manifestations of this reaction in *e*, practically all chromosomes being pulverized into some fifty fragments. Fig. 2 *g* represents a rare case of chromosome bridge, which has not been caused by mere stickiness. It is probably a translocation bridge, which is an exceptional occurrence in these treatments. After mustard gas treatment of the same material such bridges as indicate sister chromatid reunion are one of the commonest changes met with (STEINEGGER and LEVAN, unpublished). Fig. 2 *f* and *h* are late anaphases and *i* is a telophase where two fragments have formed micronuclei and one larger fragment is loitering far off in the peripheric plasm.

Parallelly to the fragmentations and the formation of constrictions there occurs another characteristic phenomenon, which we usually record under the designation of »pseudochiasmata». It consists of the hanging together at one point of the two separating sister anaphase chromosomes, which effects a pulling out by the centromeres of the portions proximal to the pseudochiasma into thin threads, while the two chromosome parts distal to this point form a pair of transverse arms. This formation has a striking resemblance to a rod bivalent with a non-terminalized chiasma. In a few cases one pseudochiasma has been seen on each of the two arms of a separating anaphase chromosome pair, which thus assumes the appearance of a ring bivalent, remaining at the equator long after the rest of the chromosomes have reached the poles. Pseudochiasmata have been depicted by DARLINGTON and KOLLER (1947), who make the assumption that these formations are caused by lack in reproduction of small parts of the chromosomes. STEINEGGER and LEVAN (unpublished) have seen them in material treated with mustard gas and LEVAN (unpublished) has seen them in treatments with several inorganic salts. Since pseudochiasmata may already occur after a short treatment, we do not agree that they are caused by lack in reproduction. We postpone the close description of the pseudochiasmata to a later occasion, being here only concerned with

them in so far as they interfere with our fragmenting reaction. The pseudochiasmata may give rise to fragments through the purely mechanical tension of the anaphase movement. These fragments are of a characteristic appearance, however, and may be readily distinguished from our fragments, which, as mentioned, are already free before the anaphase tension has started. In certain of the experimental series described below, e. g. alizarin and naphthoresorcinol, pseudochiasmata are of frequent occurrence. In most substances they may be found scattered within our zone 3. The relation of our fragments to pseudochiasmata is in no way constant. In pyrogallol, for instance, which is one of the substances with most free fragments, only one single case of pseudochiasma has been found among the thousands of cells that have been scrutinized during the course of the investigation. Nevertheless it is somewhat hard not to think that there is a connection between those reactions. In substances where free fragments are very rare it may happen that a pseudochiasma is found. When that is the case, our experience is that if one has patience to study enough cells one can always also find attached and free fragments. We have actually used pseudochiasmata as »lead-fossils» for our reaction.

The question as to how long a minimum time of exposure is necessary for the fragments to start appearing has been studied in a couple of

TABLE 2. *Fragmentation per cent after different exposure times in 0,000·5 mol/l (concentration 8) pyrogallol.*

Time of treatment in hours	I. March 17, 1948 Number of cells	Fragmentation %	II. April 2, 1948 Number of cells	Fragmentation %
0,5	100	0	—	—
1	100	0	201	0,5
1,5	100	0	—	—
2	360	2,8	204	2,0
2,5	214	6,5	—	—
3	136	11,8	106	5,7
3,5	114	12,3	—	—
4	341	30,2	303	20,8
5	108	7,4	147	18,4
6	105	1,0	88	20,5
7	112	1,8	40	7,5
8	78	23,1	34	2,9
10	30	10,0	30	0
12	21	4,8	20	0

series of 0,000·5 mol/l pyrogallol. Table 2 indicates that the first fragments appear after one hour's treatment in the one series and after two hours' in the other one. The frequency increases to a maximum at 4—6 hours. We do not dare yet to point out any mitotic stage as especially sensitive to this reaction. Purely morphologically the reaction gives the impression of being a direct damage to the chromosomes during their contraction stages, but it is not excluded that the effect seen during these stages has been prepared during early prophase or even during resting stage. Symmetric fragments have not been found after 24 hours' treatment, which they should have been if the chromosome thread had been attacked so long in advance that it acted as single, and which is found after X-ray treatment. Symmetric constrictions occur, however, after 24 hours, which may lead back to damages during the preceding mitosis and thus represent the second mitotic generation during the treatment.

II. SPECIAL OBSERVATIONS.

In the following section of this work a systematic account is submitted of the various substances tested. For each substance the following data are provided:

a: Concentration series tested.
b: Toxicity, macroscopically estimated (i. e. soft roots).
c: C-tumour reaction zone.
d: Zone 1: strong poison effect with various agglutination phenomena.
e: Zone 2: c-mitosis.
f: Zone 3: fragmentation.

For brevity, the following letters or numbers are used for designating the various concentrations:

Designation :	A	B	C	1	2	3	4
Concentration mol/l:	1	0,5	0,2	0,1	0,05	0,02	0,01

Designation :	5	6	7	8	9
Concentration mol/l:	0,005	0,002	0,001	0,000·5	0,000·2

Designation :	10	11	12	13
Concentration mol/l:	0,000·1	0,000·05	0,000·02	0,000·01

Designation :	14	15	16
Concentration mol/l:	0,000·005	0,000·002	0,000·001

Most of the substances have been bought from Kebo, Stockholm, or Svanen, Lund. Some rare substances were kindly provided by Professor H. ERDTMAN, Stockholm, and Professor L. SMITH, Lund, for which we tender our sincere thanks.

A. PHENOLS AND QUINONES.

1. *Phenol.* — *a*: B—16. *b*: Roots soft: B (5 min.), C—1 (15 min.), 2 (4 hours), 3 (1 day), 4—5 (1,5 days). *c*: C-tumours occur from concentration 5 down through the series to 16. The reaction is, however, present only in phenol + distilled water. In controls with phenol + tapwater tumours were found only in 5—7. *d*: B—2 give strong toxic effect with agglutinated, weakly stained chromosomes. *e*: C-mitosis occurs in 2—6, in 4 almost to 100 per cent. *f*: Fragmentation is very rare. In 3 one fragment was seen within an anaphasic c-pair, in which one piece of the one chromosome was cut off. In some lower concentrations, 9 and 10, pseudochiasmata and tendencies to fragmentations were seen.

2. *Pyrocatechol.* — *a*: 1—13. *b*: Roots soft: 1—2 (4 hours), 3 (16 hours), 4—6 (20 hours), 7—10 (1 day). *c*: No certain c-tumours were observed. *d*: 1—2 have strong toxicity with agglutination of chromosomes. Also differential stainings of centromeric regions and chromosome ends were seen. In 2 mottling occurs. *e*: C-mitosis in 2—6, in 3 pronounced c-mitosis with strong chromosome contraction and marked centromeric constrictions. *f*: Fragments occurred in the following frequencies in concentrations 4—13:

Concentration No.	Number of fragments per cell							
	0	1	2	3	4	5	>5	Fr %
4	97	3	—	—	—	—	—	3
5	97	1	1	—	1	—	—	3
6	89	9	1	—	—	—	—	10
7	76	17	5	1	—	—	1	24
8	82	7	4	2	2	1	2	18
9	100	—	—	—	—	—	—	0
10	97	2	1	—	—	—	—	3
11—13	100	—	—	—	—	—	—	0

3. *Resorcinol.* — *a*: 1—16. *b*: Roots soft: 1 (2,5 hours), 2—5 (4 hours). *c*: C-tumours common in the region 7—16. *d*: Zone 1 with strong poison effect: 1—2. *e*: Zone 2: 3—5. In 3 very characteristic »bow metaphases» (LEVAN, 1945). Tendencies of c-mitosis go far down

in the series. *f*: 100—200 cells were analysed in each of concentrations 4—13, only in 4—7 have single fragments been encountered:

Concentration No.		4	5	6	7
Fr %	4 hours	1	1	2	2
	1 day	—	—	24	1

4. *Hydroquinone.* — *a*: 1—16. *b*: Roots soft: 1—2 (4 hours), 3 (2 days), 4—9 (3 days), 10 (5 days). *c*: C-tumours in 7—16. *d*: Even in 1 not instantaneous killing, but strong agglutination in 1—4. *e*: C-mitosis in 1—5, c-tendencies down to 8. In 2 the c-mitosis is of ball-type, in 3 of bow-type.

Concentration No.	Number of fragments per cell							Fr %
	0	1	2	3	4	5	>5	
5	90	9	—	1	—	—	—	10
6	75	17	4	2	1	1	—	25
7	73	11	6	4	2	—	4	27
8	17	7	5	1	—	1	—	47
9	76	14	5	3	1	—	1	24
10	83	8	4	—	—	2	3	17
11	44	2	1	—	1	1	1	12
12	96	2	1	—	1	—	—	4
13	100	—	—	—	—	—	—	0

5. *Pyrogallol.* — *a*: Concentration series: 1—16. *b*: Roots soft: 1—4 (4 hours), 5 (1 day), 6 (2 days). *c*: C-tumours scattered in 3—14. *d*: 1—2 show strong agglutination, 2 has mottled chromosomes. 3—5 have the peculiarity that extremely few metaphases and anaphases occur, while many prophases are present. This may be caused by no new cells going into metaphase during the treatment, while all old

Concentration No.	Number of fragments per cell							Fr %
	0	1	2	3	4	5	>5	
6	31	5	7	5	2	—	—	38
7	33	13	10	4	1	1	—	47
8	63	30	29	16	12	15	9	65
9	63	17	7	5	4	1	4	37
10	93	5	1	1	—	—	—	7
11	88	3	4	2	2	—	1	12
12	200	—	—	—	—	—	—	0

metaphases and anaphases have already reached telophase. *e*: C-mitosis has not been identified in 3—5 owing to lack of metaphases. In 6, where all mitotic stages occur, most divisions are normal, but some have c-tendencies. *f*: Fragments occur in the following frequencies (see p. 465, below). In 12 there were recorded 2 attached fragments, the same was the case in 13. In 14, 15 and 16 no fragments were seen.

6. *Hydroxyhydroquinone.* — *a*: 1—15. *b*: Roots soft: 1—4 (2 hours), 5 (3 hours), 6—10 (8 hours). *c*: C-tumours rare, but occurring all through the series (6, 7, 8, 15). *d*: Zone 1: 1—2, agglutination strong in 1—5. *e*: C-mitosis in 2—5, though only weak tendencies, made unclear by the strong stickiness. Also in 6 weak tendencies to c-mitosis. *f*: Fragmentation in the following cases, in each cell never more than 2 fragments:

Concentration No.	6	7	8	9	10	11	12	13	14	15
Fr %	7	6	10	14	2	0	0	1	—	0

7. *Phloroglucinol.* — *a*: 1—13. *b*: Roots soft: 1—3 (4 hours), 4—5 (1 day), the series unfortunately not tested longer than one day. *c*: C-tumours occur in 6—13. *d*: 2 gives a typical picture: metaphase and anaphase chromosomes are often stainless but show beautiful stainings of the heterochromatin. Also dissolution of the chromosome arms is found, resulting in »free» centromeres. *e*: Already in 1 tendencies to c-mitosis are found, 2 and 3 give typical bow meta- and anaphases, in 3 all mitoses are of c-type. Below 5 no pure c-mitoses occur but clear c-tendencies go further down. *f*: The following fr %'s were recorded:

Concentration No.		5	6	7	8	9	10	11	12	13
Fr %	4 hours	2	0	1	0	0	1	0	0	1
	1 day	30	20	1	0	—	—	—	—	—

Pseudochiasmata were seen in 9 and 12 after 4 hours.

8. *p-Benzoquinone.* — *a*: 1—13. *b*: Roots soft: 1 (2 hours), 2—7 (4 hours), 8 (1 day). *c*: No c-tumours were observed. *d*: 1—5 have an immediate killing action, and all show strong agglutination. Chromosomes often completely destained or mottled. *c*: C-mitosis occurs in 5—8, nowhere complete, but intermingled with normal mitoses. *f*: Fragments were seen in the following treatments:

Concentration No.	8	9	10	11	12	13
Fr %	1	6	8	12	2	0

9. *Quinhydrone.* — *a*: 1—13. *b*: Roots soft: 1—2 (2 hours), 3—7 (4 hours), 8 (1 day). *c*: 10—12. *d*: 1—5 have strong toxic effects with agglutination of the chromatin; nucleoli and heterochromatin often deeply stained. *e*: 5—10 have partial c-mitosis, often changed by stickiness. *f*: Fragments in the following:

Concentration No.	9	10	11	12	13
Fr %	1	6	8	1	9

10. *α-Naphthol.* — *a*: 3—16. *b*: Roots soft: 3—6 (1 hour), 7 (3 hours), 8 (1 day). *c*: 10—12. *d*: 3—5, chromosomes in different stages of dissolution. Differential staining of the centromeric heterochromatin in 5. *e*: C-mitosis in 6—9, 8 has complete c-effect, 9 has a strikingly high frequency of multipolar spindles. *f*: Fragments are extremely rare. In 9 and 10, however, some clear cases of fragmentation were observed. In 11 several cases of pseudochiasmata were found.

11. *β-Naphthol.* — *a*: 4—16. *b*: Roots soft: 4—5 (30 min.), 6 (1 hour), 7 (1 day), 8 (2 days). *c*: Small c-tumours in 9—10. *d*: 4—5. *e*: C-mitoses in 6—8, in 8 extremely many multipolar anaphases. *f*: No fragments and no pseudochiasmata observed.

12. *Naphthoresorcinol.* — *a*: 1—13. *b*: Roots soft: 1—2 (instantly), 3 (5 min.), 4—5 (30 min.), 6—8 (1 day). *c*: Tendencies to c-tumours in 9. *d*: 1—4. In 1—2 nuclei and chromosomes agglutinated, in 3—4 thin, slender chromosomes with clear internal structure. *e*: C-mitoses begin to appear in 5 and go down to 8. *f*: Undoubted fragments occur in a couple of treatments, (6—13 after 4 hours and 9—10, 13 after 1 day were analysed): 5 % in 8, 1 % in 10 after 4 hours. In addition some cases of attached fragments and pseudochiasmata.

13. *Alizarin.* — *a*: The concentration zone tested starts with $0{,}012$ g/100 cc, which corresponds to $0{,}000^{\cdot}5$ mol/l. This was dissolved when heated. On cooling, precipitate was formed both in this solution and in the next lower one. Roughly counted, the concentration zone employed corresponds to our numbers 8—15, the point of saturation lying between 9 and 10. *b*: Roots soft: 8—9 (1 day). *c*: No c-tumours. *d*—*e*: Neither immediately killing zone nor signs of c-mitosis were seen. These zones would evidently be situated above the saturation point of alizarin. *f*: Fragmentation is very common in 8—10, i. e. in saturated and nearly saturated solution. In 11 no real fragments occur but often pseudochiasmata. The fragmentation reaction is of a special type in this substance, owing to stickiness being very common in zone 3. Ordinary

stickiness (agglutination of matrix) may lead to free fragments of chromatic substance. These are easily distinguished from our fragments, however. Fragments caused by pseudochiasmata also occur as well as fragments which seem to be formed independently by stickiness and pseudochiasmata.

Usually one type of reaction predominates within the same root, while different roots of the same treatment may represent different types of reactions. As an instance of this, the records of all roots in 4 hours' treatment in 8 are given separately:

Root No.	Number of fragments							Completely agglutinated anaphases	Fr o/o
	0	1	2	3	4	5	>5		
1	17	1	—	—	—	—	—	3	6
2	9	—	—	1	—	—	2	5	25
3	—	—	—	—	—	—	3	21	100
4	—	1	—	1	—	—	—	34	100
5	9	11	9	4	1	2	—	8	75

In roots Nos. 1, 2 and 5 varying frequency of fragmentation is present, while in 3 and 4 most divisions are agglutinated, many bridges, pseudochiasmata and loose fragments being present between the anaphase groups.

14. *Phenanthrenequinone.* — *a*: From saturated solution with excess of undissolved substance down to 0,05 saturated, which coarsely corresponds to 5—9. *b*: Roots soft: 5—6 (1 day). *c—f*: No c-tumours and no c-mitoses were seen, nor any certain fragments. In 5 there were observed several cases of pseudochiasmata.

B. ALKYLATED PHENOLS.

15. *o-Cresol.* — *a*: 1—13. *b*: Roots soft: 1—2 (5 min.), 3 (4 hours), 4—6 (1 day). *c*: 9—10 single c-tumours. *d*: 1—2 immediately killing, nucleoli deeply stained. 2 has very clear prochromosomic differentiations in metaphase—anaphase. All stages of dissolution of the chromosome arms. *e*: 3 begins to show c-mitotic tendencies, 4 has full c-mitosis with mottled chromosomes, 5 has intermingled c-mitoses and normal mitoses. *f*:

Concentration No.	6	7	8	9	10	11	12	13
Fr %	2	1	0	0	0	1	0	0

16. *m-Cresol.* — *a*: 1—13. *b*: Roots soft: 1—2 (instantly), 3 (1 hour), 4 (4 hours), 5 (2 days). *c*: Scattered c-tumours in 6—12. *d* and *e*: Agree closely with preceding substance. Very fine centromeric stainings in 2 and 3, c-mitosis in 4—5, weak tendencies to c-mitosis in 3 and 6. 4 is characterized by star and ball metaphases and by the typical mottling of the chromosomes. *f*: Fragmentation has only been observed in 5, where 1 fragment occurred in 100 cells studied.

17. *p-Cresol.* — *a*: 1—13. *b*: Roots soft: 1—2 (instantly), 3 (1 hour), 4 (4 hours), 5 (2 days). *c*: 6—11. *d* and *e*: As in preceding two substances. Stained centromere heterochromatin in 2, c-mitosis 3—5, 4 has mottled chromosomes. *f*:

Concentration No.	6	7	8	9	10	11	12	13
Fr %	4	5	0	0	1	1	0	0

In 11 there occurred one case of interlocked ring chromatids + fragment in one anaphase. In 12 there were seen pseudochiasmata.

18. *3,5-Xylenol.* — *a*: 1—13. *b*: Roots soft: 1—4 (instantly), 5 (1 day). *c*: No c-tumours seen. *d*: Zone 1: 1—4. Often distinct chromosome structures, heterochromaty of the prochromosomes very pronounced. *e*: C-mitosis in 4—7. 4 has only weak tendencies to c-mitosis, the metaphasic chromosomes are but little contracted and are often stained only at the centromeres. In 5 the c-mitosis is more typical with strong chromosome contraction and pronounced mottling. *f*:

Concentration No.	6	7	8	9	10	11	12	13
Fr %	6	0	2	0,5	0	3	1,5	0

After 1 day no fragments were met with in 100 analysed cells in each of the concentrations 8—13.

19. *Thymol.* — *a*: 4—13. *b*: Roots soft: 4—5 (2 min.), 6 (5 min.), 7—8 (1 day), 9 (2 days). *c*: Tendencies to c-tumours in 13. *d*: Zone 1: 4—6. Chromosomes faintly stained, often with distinct internal structure, showing divided chromatids. *e*: C-mitosis in 6—9, in 7 full c-mitosis with strongly contracted chromosomes, in 7—8 mottled chromosomes. *f*: Fragments occurred but extremely rarely:

Concentration No.		7	8	9	10	11	12	13
Fr %	4 hours ...	0	0	0,3	0,7	0	0	0
	1 day	—	—	—	2	0	0	0

20. *4-Ethylpyrocatechol.* — *a*: 1—13. *b*: Roots soft: 1—5 (4 hours), 6—7 (1 day). *c*: No c-tumours. *d*: Zone 1: 1—3 with unstained to weakly stained chromosomes, in 3 typical prochromosome staining. *e*: C-mitosis in 3—7, weak tendencies in 3 and 7, complete c-mitosis in 5. 3—5 often mottled chromosomes. *f*: Fragments very rare, in the following concentrations 100—200 cells were scrutinized with the following result:

Concentration No.		7	8	9	10	11	12	13
Fr %	4 hours	0	0	0	0	0	1	0
	1 day	—	—	1	0	0	1,5	0

21. *Orcinol.* — *a*: 1—13. *b*: Roots soft: 1 (1 hour), 2—3 (4 hours), 4 (1 day). *c*: 4—7. *d*: Zone 1: 1—2, with strong agglutination. *e*: C-mitosis in 2—5 with bow metaphase and mottled chromosomes in 3 and 4. In 4 there occurred after 1 day a very characteristic type of c-mitosis with strongly contracted chromosomes, the arms of which were almost spherical. These chromosomes were of an equal mottledness with the exception of the deeply stained centromeric region. *f*: No fragments were found, but one case of pseudochiasma (in 5).

22. *Thymohydroquinone.* — *a*: 2—13, 2 and 3 were dissolved in heat but, on cooling, crystals precipitated, 4 kept clear. *b*: Roots soft: 2—3 (4 hours), 4—5 (1 day), 6 (2 days). *c*: 8—12. *d*: 2 and 3 have pycnotic, deeply stained chromosomes. *e*: C-mitosis starts in 3, 4 has exclusively ball metaphases, which develop into uninuclear telophases, out of which chromosome arms extrude all around. 5 has also complete c-mitosis, but of a more ordinary type. Chromosomes often somewhat mottled in 5. In 6—7 mixed c-mitoses and normal mitoses. *f*:

Concentration No.		7	8	9	10	11	12	13
Fr %	4 hours	0	0	1	0	1	0	0
	1 day	—	—	0	0	1	0	0

C. METHOXYPHENOLS.

23. *Guaiacol* (o-methoxyphenol). — *a*: 1—13. *b*: Roots soft: 1—2 (instantly), 3 (1 day), 4 (2 days). *c*: 6—13. *d*: Zone 1: 1—2 with weakly stained, dissolving chromosomes, often with distinct internal structure. *e*: C-mitosis only in 3—4, in the former concentration a characteristic type with scattered-out, strongly contracted c-pairs, sometimes multipolar anaphases. *f*:

Concentration No.		4	5	6	7	8	9	10	11	12	13
Fr %	4 hours ..	0	1	0	4	2	1	0	0	0	0
	1 day	—	—	—	32	—	—	—	—	—	—

24. *Hydroquinone monomethyl ether* (p-methoxyphenol). — *a*: 1—13. *b*: Roots soft: 1—2 (4 hours), 3—4 (1 days), 5 (2 days). *c*: 7—13. *d*: 1 has almost completely dissolved chromosomes, 2 has strong pycnosis. *e*: C-mitotic tendencies in 2, full c-mitosis in 3—4 with strong chromosome contraction, in 3 tendencies to mottling. 5 has mixed c- and normal mitoses. *f*:

Concentration No.		5	6	7	8	9	10	11	12	13
Fr %	4 hours	1	9	8	8	5	2	0	2	0
	1 day	—	—	—	33	0	2	0	0	0

25. *Methoxyhydroquinone*. — *a*: 1—13. *b*: Roots soft: 1—2 (4 hours), 3—4 (1 day), 5—7 (2 days). *c*: 9—13. *d*: Zone 1: 1—2. Chromosome dissolution decidedly less than in the preceding two substances. *e*: C-mitosis in 3—5, in 6 weak tendencies, in no concentration, however, complete c-mitosis. *f*:

Concentration No.		5	6	7	8	9	10	11	12	13
Fr %	4 hours	4	1	7	2	1	4	1	4	0
	1 day	—	—	0	0	—	0	0	0	0

D. AMINOBENZENES AND AMINOPHENOLS, ETC.

26. *Aniline*. — *a*: C—13, *b*: Roots soft: C (instantly), 1 (1,5 hours), 2 (1 day). *c*: 3—6. *d*: Zone 1: C—1. *e*: C-mitosis in 2—3, complete in the former. *f*: Surprisingly active as fragmentation-inducing agent:

Concentration No.		3	4	5	6	7	8	9	10	11	12	13
Fr %	4 hours	3	1	4	1	0	0	1	2	5	1	0
	1 day	—	0	—	1	0	0	0	—	—	0	0

27. *o-Phenylenediamine*. — *a*: 1—13. *b*: Roots half-turgescent: 1—2 (1 day). *c*: Tendencies to c-tumours in 11—13. *d—e*: In 1 a sticky type of c-mitosis occurs intermingled with normal anaphases with bridges. 2 has c-mitotic tendencies, but most mitoses are normal. After 1 day both these concentrations have full c-mitosis in the few divisions then occurring. In similarity to its isomers this substance is characterized by showing solitary instances of typical c-mitosis far down in

the dilution series. Especially on the second day of treatment such cells are not infrequently found. *f*:

Concentration No.		3	4	5	6	7	8	9	10	11	12	13
Fr %	4 hours	3	1	0	0	0	0	0	2	2	0	0
	1 day	—	—	—	10	—	0	0	4	2	0	0

Thus, only few cases of undoubted fragments were recorded. But a pronounced stickiness extends all through the series, obscuring the diagnosis of fragments. Several concentrations held pseudochiasmata and attached fragments.

28. *m-Phenylenediamine.* — *a*: 1—13. *b*: All roots were turgescent after one day, 3—13 after 2 days, unfortunately 1—2 were not observed longer than one day, when all roots were fixed. *c*: 6—9 have tendencies to c-tumours. *d* and *e*: Some c-mitoses occur in 1, mixed with normal mitoses. After 1 day 2 also shows c-mitotic characteristics, mainly strong chromosome contraction, though all anaphases are normal. *f*:

Concentration No.		2	3	4	5	6	7	8	9	10	11	12	13
Fr %	4 hours	5	16	5	0	2	0	1	0	2	0	0	0
	1 day	—	0	6	2	10	5	0	0	0	0	0	0

29. *p-Phenylenediamine.* — *a*: 1—13. *b*: Roots soft: 1 (4 hours), 2—3 (1 day), 4 (2 days), thus more toxic than the o- and m-form. *c*: 7—13, decidedly more developed c-tumours than the preceding two substances. *d*: Strong pycnosis in 1. *e*: In 2 there are found very sticky c-mitoses, which after 1 day all have ball type. *f*: One of the most active substances, and therefore the detailed fragmentation values are given here:

Concentration No.	Number of fragments	4 hours								1 day							
		0	1	2	3	4	5	>5	Fr%	0	1	2	3	4	5	>5	Fr%
	4	99	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—
	5	91	4	3	1	1	—	—	9	47	2	1	—	—	—	—	6
	6	175	11	11	2	1	—	—	12,5	49	1	—	—	—	—	—	2
	7	64	21	10	2	—	1	2	36	43	3	2	1	1	—	—	14
	8	81	12	4	2	1	—	—	19	46	3	—	—	—	1	—	8
	9	45	2	2	1	—	—	—	10	46	1	1	—	—	—	2	8
	10	99	—	1	—	—	—	—	1	49	1	—	—	—	—	—	2
	11	99	1	—	—	—	—	—	1	49	1	—	—	—	—	—	2
	12	100	—	—	—	—	—	—	0	100	—	—	—	—	—	—	0
	13	100	—	—	—	—	—	—	0	100	—	—	—	—	—	—	0

30. *p-Aminophenol* (rodinal). — *a*: 2—13. *b*: Roots turgescent in all concentrations. *c*: 6—13. *d* and *e*: Only in 2 were c-mitoses found after 4 hours. Clear tendencies to c-mitosis were seen after 1 day in one root treated with concentration 5. No divisions occurred in adjacent concentrations, so the finding could not be verified. *f*: Fragments common, their frequency distribution resembles that of p-phenylenediamine:

Concentration No.		4	5	6	7	8	9	10	11	12	13
Fr %	4 hours ..	8	1	5	8	22	7	3	2	2	1
	1 day	—	—	—	—	—	8	28	17	1	0

31. *2,4-Diaminophenoldihydrochloride* (amidol). — *a*: 1—16 (2 series *a* and *b*). *b*: Roots soft: 1—6 (1 hour), 7—8 (4 hours), 9 (1 day). *c*: 10—13 (*a* series), 12—16 (*b* series). *d*: The entire upper region, 1—6, shows pronounced toxicity with instant breaking of the vital processes. In 6 isles with vital structures and fine chromosome structures begin to appear. *e*: No concentration gives typical c-mitosis, 7—8 show weak c-tendencies together with a certain amount of mottling. *f*: Fragments rarely occur in 8—13. Although in 3 series 100 cells in each of the concentrations 14—16 were analysed, no fragment was seen in these concentrations.

Concentration No.		8	9	10	11	12	13
Fr %	2 hours *b*	3	4	3	0	2	—
	4 » *a*	—	1	10	7	2	1
	4 » *b*	—	4	4	1	2	2
	1 day *a*	—	—	—	0	0	0
	1 » *b*	—	0	0	0	1	—

32. *p-Methylaminophenolsulphate* (methol). — *a*: C—13. *b*: Roots soft: C—3 (4 hours), 4—5 (1 day). *c*: 9—13, *d* and *e*: C and 1 have pronounced pycnosis. 2—5 show tendencies to c-mitosis, but never very strong. *f*:

Concentration No.		4	5	6	7	8	9	10	11	12	13
Fr %	4 hours ..	1	—	6	6	0	7	2	0	0	0
	1 day	—	—	—	—	—	40	0	0	0	0

33. *p-Hydroxyphenylglycine*. — *a*: 3—13. *b*: Roots soft: 3—6 (1 day). *c*: 7—8. *d* and *e*: After 4 hours 3 shows no poison effect, after 1 day all vital structures are completely broken down in 3—5. *f*:

Concentration No.		4	5	6	7	8	9	10	11	12	13
Fr %	4 hours ..	0	3	4	0	3	1	0	0	0	0
	1 day	—	—	17	12	0	9	17	0	3	0

Attached fragments were recorded in 10, 11 and 12, pseudochiasmata in 4, 5 and 13.

E. NITROPHENOLS.

34. *o-Nitrophenol.* — *a*: 1—13. *b*: Roots soft: 1—2 (1 hour), 3 (4 hours), 4—5 (1 day), 6—7 (2 days). *c*: 8—12. *d*: Strong toxicity in 1—2, in 2 vital structures with heterochromaty of the centromeric region, this being especially distinct on the second day. 1—5 have pronounced stickiness. *e*: C-mitosis in 2—6, in 5 complete c-mitosis of bow-type. *f*: Fragmentation only in isolated cases:

Concentration No.		6	7	8	9	10	11	12	13
Fr %	4 hours	0	0	0	1	0	1	0	0
	1 day	—	—	0	0	—	1,5	0	0

35. *m-Nitrophenol.* — *a*: 1—13. *b*: Roots soft: 1—2 (instantly), 3 (30 min.), 4 (4 hours), 5 (1 day), 6—7 (2 days). *c*: 10—11. *d*: Zone 1: 1—3, strong stickiness 1—6. In 1—2 dissolution of chromosomes with distinct prochromosomic differentiations. *e*: C-mitosis in 4—7, traces of c-mitosis also in 8. 6 has fairly complete c-mitosis of bow-type. *f*:

Concentration No.		8	9	10	11	12	13
Fr %	4 hours	0	0	0	1	0	0
	1 day	2	—	0	0	—	0

36. *p-Nitrophenol.* — *a*: 1—13. *b*: Roots soft: 1—2 (instantly), 3 (30 min.), 4 (4 hours), 5 (1 day), 6—8 (2 days). *c*: 9—12. *d*: Strong toxicity: 1—3, stickiness: 1—8. In 5 the chromosomes are often mottled, in 6 stained chromatin drops are formed from the outlines of the chromosomes. *e*: C-mitoses in 3—9, everywhere mixed with normal ones. *f*: Fragment seen only in one case (12) and here possibly attached.

37. *Picric acid.* — *a*: 1—13. *b*: Roots soft: 1—7 (1 hour), 8 (4 hours), 9 (1 day). *c*: 10—12. *d*: Strong toxicity: 1—3, stickiness occurs almost throughout the whole series (1—9). *e*: C-mitosis is found in 4—6, complete in 4—5. Strangely enough, below this region in 6—8 there is great destruction of the vital structures. In 6 the external layers of the roots show clear prochromosomic differentiations. *f*: Fragments

were encountered only in 12 after 4 hours, when 2 cells out of 200 had each 1 fragment, while 1 cell had 2 fragments.

F. HALOGEN PHENOLS, ETC.

38. *2,6-Dichlorophenol.* — *a*: 1—13. *b*: Roots soft: 1—4 (4 hours), 5—8 (1 day). *c*: 9—12. *d* and *e*: In 1—7 all divisions form deeply stained pycnotic lumps. This passes in 8 directly over into a more typical c-mitosis, also 9 has scattered c-mitoses, but mostly normal ones. *f*:

Concentration No.		9	10	11	12	13
Fr %	4 hours	0	4	0	0	0
	1 day	—	3	0	—	0

39. *Tribromophenol.* — *a*: 10—13. Only these four concentrations were tested, and the determination of the concentrations is rather approximative. *b*: Roots soft: 10 (4 hours), 11 (1 day). *c*: 12—13. *d* and *e*: C-mitosis in all concentrations, complete in 10 and 11, mixed with normal mitoses in 12, only c-tendencies in 13. *f*: In 12 and 13 100 cells were analysed; no fragments were found.

40. *Gallic acid.* — *a*: 1—13. *b*: Roots soft: 1—2 (30 min.), 3—4 (1 hour), 5—6 (4 hours). *c*: 7—8. *d*: Strong toxicity in 1—4. In 3—4 the outer layers of the roots contain good chromosome structures with heterchromatic differentiations. *e*: 5—6 give some small tendencies to c-mitosis. *f*: The concentrations 7—13 were analysed both after 4 hours and 1 day. Only in one concentration (8) after 4 hours were any free fragments met with, viz. one cell with 1 fragment and one cell with 2 fragments. Attached fragments and pseudochiasmata were sometimes seen.

41. *Purpurogalline.* — *a*: This substance has one condensed 6- and one 7-ring, around which one keto- and four hydroxy-groups are placed in the positions 1, 2, 7, 8 and 9. It is only sparingly soluble in water, 15 mg did not dissolve on boiling in 15 cc, but this solution was used for a dilution series, which approximately corresponds to our concentrations 5—11. From 7 downwards the solutions were clear. *b*: Roots soft: 5—7 (1 day), 8 (2 days). *c*: 9. *d* and *e*: Ball c-mitoses occurred in 5—7, the rest had normal mitoses. This substance, thus, shows no specific c-mitotic activity. Substances which, like colchicine, contain 7-rings, are of special interest in respect of c-mitosis. *f*: In 7 and 8 several pseudochiasmata and bridges were seen. Free fragments occurred in the following frequency:

Concentration No.		8	9	10	11
Fr %	4 hours	2	2	0	0
	1 day	—	3	0	1

42. *Cyclohexanol.* — *a*: 1—13. *b*: Roots soft: 1 (2,5 hours), 2 (1 day). *c*: 3—6. *d* and *e*: In 1 and 2 pronounced stickiness is present. In 2 a very characteristic picture results: All arms of the separating anaphase chromosomes have thick terminal portions, which evidently consist of agglutinated matrix substance floating out towards the chromosome ends. In 1 and 2 there are found centromeric differentiations. These concentrations also have clear c-mitoses, although only partial. *f*: Cyclohexanol is very inactive, but nevertheless more undoubted free fragments were found than in phenol.

Concentration No.		3	4	5	6	7	8	9	10	11	12	13
Fr %	4 hours	0	1	0	0	0	0	1	1	0	0	0
	1 day	0	0	0	0	0	—	—	1	—	—	0

III. CONCLUSIONS.

The substances employed in this investigation are of a somewhat different type from the indifferent c-mitotic substances. They all contain reactive groups, hydroxy, keto or amino. The resemblance in their effect on living matter is striking. In high concentrations they have an immediate killing action. This zone 1 passes over into the c-mitosis zone 2, and further below this zone the fragmenting concentrations start. Zone 1 extends from saturated concentration down to concentrations at which the cellular death is retarded long enough to allow the c-mitosis to be manifested. Any close analysis of the c-mitosis met with here falls outside the scope of the present paper, only a few facts characteristic of the c-mitosis in such substances as those here employed will be discussed.

As is well known, c-mitosis is a typical threshold reaction; it does not occur below a certain concentration. The threshold is not always sharp, however, and in some of the substances concerned in this study single c-mitoses and c-tendencies may be found far below the concentration which must be considered the threshold concentration. In most of the ordinary c-mitotic substances of the narcotics type an interesting correlation has been found between their activity thresholds and certain physical properties, above all their water solubility. Usually the activity

does not go further down below the water solubility than that the relation between these two concentrations is 1 : 10. In fact, this *threshold concentration* : *water solubility* relation gives an indication of the mode of action of a substance. If this relation (roughly representing the thermodynamic activity, as introduced into the colchicine work by GAVAUDAN and co-workers) is less than 0,05 it is to be suspected that the mechanism of action is not purely physical but that certain chemical reactions are also involved. Thus, in *Allium* roots colchicine has a very low threshold value and its thermodynamic activity (hereafter abbreviated »th. a.») is $0{,}000^{\cdot}3$. STEINEGGER and LEVAN (1947 a, 1948) and STEINEGGER (1947) have shown that the least change in the ring *C* of the colchicine molecule eliminates its chemical activity. *Iso*-colchicine and colchicëine have th. a.'s of 0,28 and 1 respectively. Still, if colchicëine is changed into trimethyl-colchicinic acid by the removal of an acetyl and uncovering of an amino group, fresh chemical activity is acquired.

The substances worked with in this study, the phenols, as well as the amines, may be expected to have chemical activity. Phenol was tested by GAVAUDAN, DODÉ and POUSSEL (1945), and turned out to have a th. a. of 0,005. The same authors also record aniline as an instance of chemically active substances (th. a. 0,038).

LEVAN and ÖSTERGREN (1943, Fig. 2) and ÖSTERGREN (1944, Fig. 6) have drawn up correlation diagrams of c-mitotic thresholds and water solubility in the benzene and the naphthalene groups. A distinct correlation was found between falling thresholds and falling water solubility. If a similar diagram is set up for the present substances, it is found that there is a very clear correlation present, and, if the values from the earlier papers are inserted into the same diagram, it is seen that the new substances are grouped along the same correlation line. The spreading around the correlation line is, however, very pronounced, so the correlation of the present substances is considerably looser than that of the earlier ones. Especially many variates are pulled downwards in the right part of the diagram, which means that the activity threshold is too low in relation to the solubility, i. e. the th. a. is low, which indicates chemical action.

It is quite striking that certain groups of substances have about the same threshold, regardless of the solubility. Thus, the phenols have thresholds at about 5—7, while their solubility (according to HODGMAN, 1944) varies considerably. They all have a low th. a.:

Phenol	0,002
Pyrocatechol	0,000·5
Resorcinol	0,000·2
Hydroquinone	0,001
Pyrogallol	0,000·4
Hydroxyhydroquinone	$<$ 0,05
Phloroglucinol	0,005

One methyl group introduced into phenol does not change the th. a., both the threshold and the solubility being, broadly speaking, the same:

o-Cresol	0,001
m- »	0,004
p- »	0,001

If, on the other hand, two methyl or one methyl and one isopropyl group are added, as in xylenol and thymol respectively, the water solubility is lowered and also the threshold value, which results in a th. a. of 0,001 in xylenol and 0,02 in thymol. The di-phenol homologues ethyl pyrocatechol, orcinol and thymohydroquinone have about the same water solubility with a somewhat higher threshold, which gives a th. a. of 0,02—0,1. If cresol is compared with orcinol and thymol with thymohydroquinone, the th. a. is increased in both cases simultaneously as OH is added. Thus, there is no simple connection between the OH content of a molecule and its chemical action.

The three methoxyphenols studied, guaiacol, p-methoxyphenol and methoxyhydroquinone have a solubility of about 1 and a threshold at 4—6. Their th. a. will be 0,1, 0,05 and 0,02 respectively. So in this case the introduction of another OH increases the chemical action.

The above data do not aim at anything but a description of the gathered material. Owing to the great sources of error from which, as we are well aware, our experiments suffer we consider it necessary to repeat the experiments and to take into more detailed account such factors as the purity of the chemicals, the properties of the water used, the pH of the different solutions, the temperature, and so on, before we venture to consider the results as having enough strength for the building of any hypothesis on them.

The main object of the present work is the study of the interesting new fragmenting effect. The distribution of the fr % in different concentrations of the same substance is striking, it increases from both

sides to a maximum. Since the left part of this curve is probably a function of two counteracting tendencies, one favouring fragmentation and one checking it through some form of toxic action, only the right part of the curve, from the maximum down to the lowest concentration, can be considered to give a significant representation of the activity of the substance in question. The curves have the following appearance in some of the most active substances:

Concentration No.	4	5	6	7	8	9	10	11	12	13	Mean concentration
1. Pyrogallol	—	—	38	47	65	37	7	12	0	0	7,8
2. Hydroquinone	—	10	25	27	47	24	17	12	4	0	8,0
3. Hydroxyhydroquinone .	—	—	7	6	10	14	2	0	0	1	8,0
4. Benzoquinone	—	—	—	—	1	6	8	12	2	0	10,3
5. p-Phenylenediamine ...	1	9	13	36	19	10	1	1	0	0	7,6
6. p-Aminophenol	8	1	5	8	22	7	3	2	2	1	7,8

While Nos. 1—3 and 5—6 are in fair agreement, No. 4, benzoquinone, deviates somewhat. This deviation, however, may be due only to the toxicity of higher concentrations being more pronounced in this substance. The values of the concentrations 10—13 are in good agreement with these values in the other substances.

The distribution of values in the descending part of these curves is of interest. There is a fairly good proportionality between concentrations and fr %:

Conc. mol/l × 10^{-6}:		1000	500	200	100	50	20	10
Pyrogallol	found	—	65	37	7	12	0	0
	expected ..	—	69	28	14	7	3	1
Hydroquinone	found	—	47	24	17	12	4	0
	expected ..	—	65	26	13	6	3	1
p-Phenylenediamine	found	36	19	10	1	1	0	0
	expected ..	36	18	7	4	2	1	0
p-Aminophenol	found	—	22	7	3	3	2	1
	expected ..	—	21	8	4	2	1	0

Even if the agreement is not especially good in all series, there is no doubt that the values fit better into a direct proportionality than, for instance, into an exponential proportionality.

This situation certainly indicates that this fragmentation reaction has a status different from that of the ordinary narcotic reactions, such

as c-mitosis, c-tumours, camphor-reaction of yeast, etc. In the fragmentation it is not the question of a threshold reaction but of a reaction manifesting itself in proportionality to the number of molecules of the active substance present. The active zone of all substances stretches about equally far down. Most of the substances have a very low activity, which does not mean that their threshold is high, but that their fr % is low in the entire zone. Cyclohexanol, for instance, which is of very low efficiency, exhibits in the analysed zone, 3—13, 1 fragment in each of the concentrations 4, 9 and 10. This undoubtedly shows some resemblance to the strange type of c-mitosis occurring in some of the substances studied, e. g. in o-phenylenediamine, where c-mitosis is present partly in the highest concentrations, 1 and 2, and partly in some very solitary cases in the lowest concentrations, 9 and 11. In o-nitrophenol, where the threshold is situated at 6, the presence is recorded of perfectly typical single c-mitoses as far down as in 11.

The very morphology of the fragmentations indicates that an attack is made towards certain points of the chromosomes. This can evidently result in different ultimate effects, free fragments or secondary constrictions. Whether the pseudochiasmata are also to be included in this complex of reactions, or whether they constitute quite another effect, is not known. The figures of Table 1 as well as some data given in the special chapter give instances of the frequency of free fragments occurring per cell. This shows that while cells with 0 fragments are commonest their frequency falls from 1—5 fragments per cell. An interesting observation which does not appear in these data is the presence of single cells with very many fragments, 30—50. In fact, the entire chromosome set can be completely blown up (Fig. 2 *e*). A similar condition is also valid in the case of the induced constrictions (Fig. 2 *a*). Besides the normally expected constrictions, one to a few per cell, there will be found certain cells in which all chromosomes are overheaped with constrictions, the chromosomes appearing like strings of beads. If the single fragments and constrictions may be regarded as point-effects in which each damage corresponds to one localized chemical attack, these pulverized or eroded cells must be considered as due to some other effect. It seems as though the entire cellular physiology in these cases has been switched over into an off-road.

On trying to make a comparison of the tested substances according to their fragmentation activity one can consider (1) the number of concentrations with fragments, (2) the maximal fr % of each substance.

Among the substances which exhibit fragments in more than half of the tested concentrations the following have the highest fr %:

	Highest fr %
1. Pyrogallol	65
2. Hydroquinone	47
3. p-Phenylenediamine	36
4. Pyrocatechol	24
5. p-Aminophenol	22
6. Hydroxyhydroquinone	14
7. Benzoquinone	12

This list is rather significant in one respect: Common to Nos. 1—6 is the fact that they are readily oxidized. No. 7 may in the living cell form a system hydroquinone ⇄ paraquinone. The list includes all those phenols which are most easily oxidizable, viz. those which have their OH in ortho- or para-position. Phenol, resorcinol and phloroglucinol are decidedly less active, even though they all give some fragments. All these facts go to show that the activity is in some way connected with the oxidation of the substances. Either they may interfere at some significant point with the normal oxidation-reduction system of the living cell, or their action is brought about by some product arising at their oxidation, consequently a substance being more active the easier this product is formed.

The present fragmentation reaction is strikingly reminiscent of X-ray effects and mustard gas effects. Our preliminary results indicate, however, that considerable differences are also present. Above all, it seems that the induction of translocations (reunion of broken ends) is much rarer in our reaction than in X-ray treatments. This may be due to the chemically induced breaks of the chromosome thread being spread over a long period while the X-ray breaks appear simultaneously. Broken ends have a greater chance of finding each other in the latter case. Treatments with mustard gas (STEINEGGER and LEVAN, unpublished) within the same material show that translocations formed a large fraction of the induced changes. The changes induced in *Allium* root tips by mustard gas did not at all reach the same frequency as the fragmentations in our most active substances. It is evident that these high frequencies of profound destruction of the chromosome material, without killing of the cell and tissue treated, make our substances highly interesting in relation to the cancer problem as well. PARMENTIER

and DUSTIN (1948) have shown that hydroquinone can be injected into mice intraperitoneally or subcutaneously in doses strong enough to give a cell reaction evidently identical with our fragmentation reaction. A testing on mice of some of our most active substances is planned.

SUMMARY.

About forty substances containing phenolic OH or (and) NH_2 have been tested with the *Allium*-test as to their activity in inducing chromosome fragmentation. It was found that almost all of them had some activity, although generally a rather low one. The following substances were most active: pyrogallol, hydroquinone, p-phenylenediamine, pyrocatechol, p-aminophenol, hydroxyhydroquinone, benzoquinone. In the most active substances more than 60 % of the treated cells may contain fragments.

The fragments were of two types: Completely free fragments, which were left in the equator region at anaphase, and attached fragments, which were hanging together with the rest of the chromosome by a thin thread. In addition, there occurred fragments due to stickiness, these probably being only chromatin drops, and fragments due to the breaking off of the chromosome thread between a centromere and a »pseudochiasma». In a few cases sister chromatid reunion was demonstrated, but, on the whole, translocations were extremely rare.

The chromosome fragmentation occurred in a concentration zone starting at 0,05—0,02 mol/l in some substances and lower in other substances, reaching down to $\pm$ 0,000·01 mol/l. Above this zone there occurred in almost all substances some concentrations showing c-mitosis and further above this zone there occurred strong toxic effects with various agglutination phenomena. The c-mitotic threshold was often situated further down than 0,05 of the water solubility concentration, which indicates that many of these substances induce c-mitosis by a specific chemical action.

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CYTOLOGICAL DISTURBANCES IN THE FIRST INBRED GENERATIONS OF RYE

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I. INTRODUCTION.

IN 1936 LAMM reported the occurrence of rather pronounced meiotic irregularities in lines of rye (*Secale cereale*) which had been inbred for about eight generations. On an average the chiasma frequency was lower in the inbred material than in the population plants, and in some lines non-conjunction and ensuing irregularities were found to be frequent. Other characteristic deviations from the normal course of meiosis were a reduced contraction of the chromosomes and at first anaphase difficulties of separation and fragmentation.

The same rye material, originally raised by H. NILSSON-EHLE, was again investigated by R. PRAKKEN and A. MÜNTZING in the years 1939—1941, the lines at that time being inbred for about 15 generations. Some special results of this investigation were reported in 1942 and the main bulk of data will be published by Dr. PRAKKEN. It seemed important not only to study meiosis in material which had been inbred for many generations but also to examine the very first generations of inbreeding. Hence, a new material was raised in 1941, by isolation of population plants of »Stålråg», the same variety as previously used. As usual, seed setting in the isolation bags was very poor but in 49 plants at least one seed was produced, the total number of seeds obtained being 301. These seeds were sown in the field in the autumn of 1941, and in the following summer 130 I_1 plants were available, these plants representing 20 different lines. As far as possible the I_1 plants were used for fixation, studies of pollen fertility and for new isolations. After the harvest plant height, number of tillers and number of seeds in the isolation bags were determined. Altogether 975 seeds were obtained and sown in the autumn of 1942. This resulted in 190 fullgrown I_2 plants, studied in the following summer and representing 13 different lines. The I_2 plants produced a total of 2131 seeds in the bags, and this gave rise to 259 I_3 plants in the summer of 1944. These plants belonged to 11 different lines.

At this stage it was found to be impossible to follow the entire material in the same way as before and in I_4 only some special fixations were undertaken. Thus, most of the data given in this paper are based on the first three generations of inbreeding. Besides the inbred material population plants of Stålråg were grown as controls and were fixed as far as possible at the same time as the inbred plants.

The cytological analysis was not worked out until recently when one of us, AKDIK, had an opportunity to carry out this work. All fixations were made in chrome-acetic-formalin following prefixation in Carnoy. The slides were stained with crystal violet or in some special cases according to the Feulgen technique.

II. DEGREE OF CHROMOSOME PAIRING IN I_1—I_3.

1. AVERAGE VALUES OF THE GENERATIONS.

As it seemed more important in this case to supervise a relatively large number of plants than to make detailed studies of chiasma frequencies, the degree of chromosome pairing at first metaphase of meiosis was merely measured by counting the number of paired chromosome ends. This number may range from 0 to 14, the extreme values representing complete asyndesis and cells with 7 ring bivalents respectively. Intermediate values correspond to metaphases containing rod bivalents or univalents or both. The »pairing value» of each plant represents the average value of 50 p. m. c.'s. At the same time the average number of univalents was determined. This number, however, is of less interest than the pairing value.

The interplant variation in degree of pairing is very obvious in the inbred material, and even among the population plants some individuals may be clearly deviating. At present there is no reason to publish the extensive data showing the variation within each plant and to compare intra- and inter-plant variation. Thus, in the following table (Table 1) the variates represent the average pairing values of the plants examined.

According to Table 1, the average pairing value of the I_1 plants examined is 12,71, the corresponding value of the population plants studied at the same time being 12,99. The significance of this difference was tested with the χ^2-method. Adding the scattered variates with pairing values lower than 13,0 together into one class, the distributions to be compared will be 6—18—5 (pop. plants) and 22—20—10 (I_1 plants). χ^2 will be 4,77 and P intermediate between 0,10 and 0,05.

Thus, though a difference is indicated it is not large enough to be significant.

In I_2 the average pairing value, 11,69, is again lower than the corresponding value (13,25) of the population plants. The simplest way to test the significance of this difference is to divide the long and variable I_2 series into two parts having approximately the same number of variates and to compare the distribution thus obtained with the corresponding distribution of the population plants. If this is done, the classes 1,0—12,5 and 12,5—14,0 in the two series will give the distributions 0—11 and 49—41 respectively. A χ^2-test gave the value 11,63 of χ^2, which corresponds to a P smaller than 0,001. Thus, there is no doubt that chromosome pairing at I—M is less good in I_2 than in the control plants. I_2 is also characterized by a very marked variation, the pairing values representing most classes from 1,0 to 14,0.

Contrary to expectation the difference between population plants and the inbred material was less pronounced in I_3 than in I_2, the average pairing values being 12,96 and 12,23 respectively. Testing the difference in the same way as in I_2, the distributions to be compared will be 1—11 and 30—30. In this case χ^2 will have the value 7,08, which corresponds to a P intermediate between 0,01 and 0,001. Thus,

TABLE 1. *Chromosome pairing in I_1—I_4.*

Category	Number of chromosome ends paired at I—M																									n	M
	1,0	—	1,5	—	2	—	2,5	—	3	—	3,5	—	4	—	4,5	—	5	—	5,5	—	6	—	6,5	—	7	—	7,5	—	8	—	8,5	—	9	—	9,5	—	10	—	10,5	—	11	—	11,5	—	12	—	12,5	—	13	·	13,5	—	14		
Pop. I_1																					1		—		—		—		—		—		—		—		—		—		1		4		18		5	29	12,99
															1		—		—		—		—		—		—		—		—		1		—		2		4		7		20		10	52	12,71
Pop. I_2	1		—		—		1		—		—		—		1		—		1		1		2		1		3		2		4		15		14		29		10		2	90	11,69

also in this generation the inbred plants have less good pairing than the population plants.

It is also of interest to compare the values of the different inbreeding generations with each other. A comparison of I_1 and I_2 will be based on the following distributions:

Pairing value	1,0 — 11,5	— 12,0	— 12,5	— 13,0	— 14,0
I_1	4	4	7	7	30
I_2	20	15	14	29	12

In this case χ^2 will be 32,94 and P smaller than 0,001. Thus, there is certainly a significant difference between the I_1 and I_2 distributions.

It is of special interest to see whether the apparent increase in pairing from I_2 to I_3 is significant. The series to be compared will be:

Pairing value	1,0 — 11,5	— 12,0	— 12,5	— 13,0	— 14,0
I_2	20	15	14	29	12
I_3	6	7	17	22	8

This gives a χ^2 value of 6,78 corresponding to a P intermediate between 0,20 and 0,10. Thus, the difference is not significant. Anyhow, it is very striking that the decrease in pairing which was initiated in I_1 and conspicuous in I_2 was interrupted in I_3 or even turned in the other direction. This observation is further supported by the fact that the average value of the population plants fixed at the same time as I_3 is lower rather than higher than the average value of the population plants corresponding to I_2. This indicates that a selection favouring plants with good pairing has really occurred. This selection may occur at various stages. It may be recalled that the percentages of fullgrown plants per number of seeds sown were rather low. As mentioned in the previous paragraph, the number of seeds and plants were the following in the three generations:

	Number of seeds sown	Number of mature plants	Plant percentage
I_1	301	130	43,2
I_2	975	190	19,5
I_3	2131	259	12,2

The plant percentages show a decrease from I_1 to I_3 and are on an average rather low, thus allowing of a marked selection. It might also be supposed that the I_3 material had descended from I_2 mother plants

having pairing values higher than the average. This was not the case, however. Of the I_3 material 37 plants had a total of 21 mothers with known pairing values. These mothers had the average pairing value 10,93, which is not higher but lower than the total average of I_2 (11,69). The average of the I_3 daughters of these 21 mother plants was 11,91, which is only slightly lower than the average of the entire I_3 generation (12,23).

Another possible cause of changes in degree of pairing might be differences in the environmental conditions between years and even between different days of fixation. From the works of MATHER and LAMM (1935) and of LAMM (1936) it is known that such environmental changes may have a very marked influence on chiasma frequencies and chromosome pairing. However, comparing firstly the different years, we find that the population plants have about the same average values (12,71, 13,25 and 12,96). The possible occurrence of a significant heterogeneity was tested with an analysis of variance, but the result was entirely negative ($v^2 = 0{,}59$). As the population plants represent the same average genotype each year, it may be concluded that the average differences in pairing between the inbred generations are not significantly influenced by environmental differences.

Though the differences between the generations are not seriously affected by environment, it might be suspected that changes in environmental conditions during the period of fixation might tend to increase the variation in pairing value within each generation. This possibility is elucidated by the data given in Table 2. According to this table, the I_1 plants and the corresponding population plants were fixed on 8 occasions during a period of 13 days. Considering the low number of plants, the average pairing values on the different days of fixation are very similar and do not indicate any important fluctuations caused by environmental influences. The same thing may be said about I_2 and I_3, possibly with one exception, viz. the I_2 plants fixed on May 19th and 20th. However, the relatively large difference between the average values (12,9 and 11,6) is caused by the fact that on the latter day some plants belonging to a family with very low average pairing value were fixed. Other plants of the same family, fixed some days later, also had poor pairing. Thus, though the influence of environmental fluctuations must be taken into consideration, the data in Table 2 strongly indicate that in the present material this source of error has been unimportant.

When the cytological material was being examined not only the

TABLE 2. *Average pairing value in different days of fixation.*

Date	Generation	Inbred plants	n	Popul. plants	n
June 3, 1942	I_1	—	—	13,3	10
» 4, »	»	12,9	17	13,2	3
» 5, »	»	—	—	13,3	6
» 8, »	»	12,8	6	13,4	2
» 10, »	»	12,7	24	12,2	6
» 12, »	»	12,9	6	—	—
» 13, »	»	—	—	12,8	2
» 15, »	»	13,0	3	—	—
May 19, 1943	I_2	12,9	18	—	—
» 20, »	»	11,6	26	—	—
» 21, »	»	11,4	34	12,8	6
» 22, »	»	11,4	5	13,3	2
» 25, »	»	11,5	2	—	—
» 26, »	»	9,8	4	13,4	3
» 27, »	»	12,2	2	—	—
May 30, 1944	I_3	12,6	17	12,5	1
June 1, »	»	12,3	9	13,1	2
» 2, »	»	12,4	20	12,7	5
» 3, »	»	11,6	10	13,4	1
» 5, »	»	11,8	4	13,0	3

number of paired chromosome ends were noted but also the frequency of univalents. They always occur in even numbers ranging from 2 to 14. Naturally there is quite a strong negative correlation between pairing value and univalent frequency, as was especially apparent in the variable I_2 generation. Since, for our present problems, the pairing value is of more interest than the univalent frequency, there is no need to give any details concerning the latter property.

2. DIFFERENCES BETWEEN LINES AND FAMILIES.

As the genotypical differentiation, commencing in I_1, may also be expected to influence the meiotic behaviour, the degree of chromosome pairing in different inbred lines and families was compared. The first thing that could be done in this respect was to compare the intra- and inter-family variation in I_1, I_2 and I_3.

In I_1 the 52 individuals examined belonged to 20 different families, the number of plants per family ranging from 1 to 11. The variation in pairing value within and between families was studied with an

TABLE 3. *Chromosome pairing in I_2.*

| Line | I.-family | Number of paired chromosome ends | | |

analysis of variance. There was no significant difference, v^2 in this case having the value 0,92.

In I_2 the 90 individuals examined belonged to 25 different families, As may be seen from Table 3, there are obvious differences in average pairing value between the families and this was verified by an analysis of variance. In this case the v^2 value was found to be 6,94, which, for 24 degrees of freedom for the variation between series and 65 degrees for the variation within series, corresponds to a P much smaller than 0,001.

In I_3 the variation was found to be less pronounced than in I_2 (Table 1) and as regards the comparison between inter- and intra-family variation no significant difference could be established ($v^2 = 0{,}69$). This is partly due to the low number of individuals per family which could be examined in this generation. In I_3 the average number of plants per family (cytologically examined) was as low as 1,7. The corresponding values in I_1 and I_2 were 2,6 and 3,6 respectively.

In I_1 all the 20 families represent separate lines of inbreeding, in I_2 and I_3 some of the families are related and derived from the same original population plant. Thus, the 25 families in I_2 belong to 13 different lines as indicated in Table 3.

In lines represented by more than one family the pairing values were added together and in this way 13 series were obtained, each corresponding to one inbred line. An analysis of variance was undertaken, and again the variation between series was found to be much greater than the variation within series, v^2 being 9,95 and P much smaller than 0,001. In the first place this high v^2 value is caused by Line 9 (Table 3), in which the average pairing value is as low as 8,00. If this series is omitted and the calculation repeated, v^2 will be 2,40 and P just slightly larger than 0,01. Thus, the rest of the material also shows a significant heterogeneity. This result demonstrates that in I_2 there was not only a significant heterogeneity between families derived from different I_1 plants but also a significant heterogeneity between lines derived from the different original population plants. In I_3 no heterogeneity between families could be demonstrated. This negative result was considered to be partly due to the low average number of individuals per family. Just as in I_2, a grouping of the families into lines was undertaken, the 35 I_3 families belonging to 11 lines. The occurrence of heterogeneity was tested with an analysis of variance but the result was again negative, v^2 being smaller than 1.

3. CORRELATION BETWEEN MOTHER PLANTS AND OFFSPRING.

The genotypical basis of chromosome pairing in the present material may be further elucidated by comparisons between the pairing values of mother plants and offspring. Unfortunately the material examined was not so comprehensive as was desirable, and in several cases data on the degree of chromosome pairing in the mother plants were not available. However, comparisons between I_1 and I_2 could be made with regard to 7 mother plants and their offspring. The pairing values of the mother plants and the average pairing values of the corresponding daughter plants are given in the first part of Table 4. For each I_2 family the number of individuals is also given, this number ranging from 3 to 17. The coefficient of correlation was calculated for the seven pairs of values and was found to be as great as + 0,72. However, a test of the significance of this value according to the formula $t^2 = \frac{(n-2)r^2}{1-r^2}$ gave a t^2 of 5,43, which for 5 degrees of freedom corresponds to a P intermediate between 0,2 and 0,05.

The same correlation was also examined with regard to I_2 mother plants and I_3 progenies. In this case 21 pairs of values were available (Table 4). For this material, in which the number of I_3 plants per family is as low as 1 or 2, the coefficient of correlation was found to have the value of + 0,26. The significance of this r-value was tested

TABLE 4. *Comparison between pairing values in mother plants and offspring.*

Mother plant		Offspring			Mother plant		Offspring		
Generation	Pairing value	Generation	Average pairing value	n	Generation	Pairing value	Generation	Average pairing value	n
I_1	11,78	I_2	7,82	10	I_2	13,06	I_3	13,26	1
»	11,78	»	11,97	5	»	12,54	»	12,70	1
»	13,46	»	12,39	3	»	9,18	»	12,98	2
»	13,51	»	12,81	3	»	12,04	»	11,03	2
»	12,76	»	11,88	4	»	12,40	»	12,92	1
»	13,58	»	12,84	7	»	12,54	»	11,96	2
»	13,24	»	12,23	17	»	13,04	»	12,58	2
I_2	8,10	I_3	12,81	2	»	11,88	»	12,39	2
»	1,30	»	10,91	2	»	12,22	»	11,65	2
»	9,90	»	8,71	2	»	13,22	»	12,68	2
»	5,34	»	12,90	2	»	12,44	»	12,28	1
»	10,76	»	12,80	2	»	12,94	»	11,86	1
»	13,44	»	13,02	2	»	12,90	»	12,17	2
»	12,86	»	12,82	2	»	12,66	»	13,07	2

as before, giving a $t^2 = 1{,}81$ and a P which is again intermediate between 0,2 and 0,05. Though each r-value obtained does not reach the level of significance, the fact that both these values are positive and nearly significant indicates that the pairing values of mother plants and offspring are really positively correlated to some degree.

TABLE 5. *Average pairing values of 13 inbred lines (I_1—I_3).*

Line No.	I_1		I_2		I_3	
	M	n	M	n	M	n
1	13,75	2	12,50	3	12,75	1
2	12,50	4	12,50	4	9,75	2
3	13,11	11	12,80	19	12,75	12
4	13,13	8	12,16	16	12,66	12
5	11,75	1	11,55	5	11,65	5
6	13,25	2	12,25	8	12,50	4
7	13,75	1	11,76	7	12,75	4
8	13,42	3	11,75	1	—	—
9	10,55	5	8,00	14	11,44	8
10	13,75	1	13,25	1	12,50	2
11	13,25	1	12,25	5	11,75	4
12	12,59	3	11,75	1	—	—
13	12,50	2	12,08	6	12,09	6

In connection with this comparison between chromosome pairing in mother plants and offspring Table 5 was elaborated. This table shows the average pairing value of the inbred lines in I_1—I_3. Thus, in I_2 and I_3 the values of different families derived from the same original population plant have been added. Lines 14—20 are only represented in I_1 and, hence, not included in Table 5. The average pairing value of these lines, 12,44, is only slightly below the average pairing value of I_1, 12,71.

The other values show a certain correspondance if the same line is followed from I_1 to I_3. In most cases, the line is either above or below the average of each generation. Thus, Lines 3 and 4 are good examples of plus lines, whereas Line 5 and especially Line 9 represent minus lines. Inter- and intra-line variation was compared by an analysis of variance. Excluding Lines 14—20, which were only studied in I_1, there remain 13 lines each represented by three (or in two cases by two) values. The following result was obtained:

	Degrees of freedom	Sum of squares	Mean square
Between lines	12	25,11	2,09
Within »	24	19,18	0,80
Total	36	44,29	—

The v^2-value (2,09 : 0,80) will be 2,61, which corresponds to a P intermediate between 0,05 and 0,01. Thus, it is rather probable that the line differences observed are really significant.

III. SPECIAL MEIOTIC ABNORMALITIES. VARIATIONS IN STAINING CAPACITY.

1. DATA FROM I_1—I_3.

So far only the degree of chromosome pairing has been considered, but, besides variations in this respect, several other meiotic abnormalities were observed. In the main these observations were confined to first metaphase and, thus, aberrations occurring at first anaphase or later were not studied. The data obtained are summarized in Table 6. Considering first the upper part of this table, giving the total values for I_1, I_2 and I_3, it may be seen that the percentage of plants showing abnormalities at meiosis (other than a decrease in pairing) increases from 17,4 in I_1 to 75,0 in I_3. The differences between the generations in this respect are significant, as may be verified by χ^2-tests.

The high proportion of plants with abnormalities in I_3 is in the first place caused by the frequent occurrence in this generation of plants with poorly stained chromosomes, as many as 47 plants of 61 showing an apparent »lack of chromatin». However, the same feature was also observed in 3 population plants, the other 9 population plants being quite normal. The occurrence of population plants as well as I_3 plants with poor staining capacity indicates that some environmental factor may contribute to this peculiarity. Nevertheless, there remains a significant difference in frequency between the I_3 and the control plants, the ratios of *normal* : *poor staining* being 14 : 47 and 9 : 3 respectively. A χ^2-test of these ratios gives a χ^2 value of 12,6 and a P smaller than 0,001. Thus, special environmental conditions are not the only cause of this phenomenon, the I_3 plants having a much greater tendency to poor staining than the population plants.

In I_2 only 5 of 90 plants showed a somewhat poor degree of staining, and all the 11 population plants were quite normal. All the I_1

plants were normal in this respect, just as were the population plants examined at the same time.

Among other deviations from normal behaviour may be mentioned poor contraction (Fig. 1) and strong contraction (Fig. 2), the former deviation probably being more frequent than the latter (cf. Table 6). Naturally there are no sharp limits between the normal degree of contraction and the plus or minus deviations. The cases represented in the table may be regarded as rather extreme ones.

Frequently the spindle was found to be obviously abnormal, or one or a few bivalents showed a bad orientation, being more or less outside the metaphase plate. Cases of the latter kind are represented by Figs. 3—4. In Fig. 16 the spindle is long and bent and situated at the side of the cell, in Fig. 17 it also has a lateral position but is quite short. Another quite characteristic deviation, which is probably again an expression of an abnormal spindle, is represented by Figs. 5—6. The rod bi-

TABLE 6. *Meiotic observations in I_1—I_3.*

Line and generation	Number of individuals	No special irregularities	Poor contraction	Strong contraction	Abnormal spindle, bad orientation of biv.	Rod bivalent not stretched	Ring orientation of bivalents	Agglutination	Lack of chromatin	Other irregularities	Per cent plants with special irregularities
I_1, total	46	38	—	—	1	—	—	—	—	8	17,4
I_2, »	90	48	8	6	16	6	8	6	5	6	46,7
I_3, »	60	15	3	2	5	5	—	1	41	3	75,0
Line 3, I_1 ...	12	9	—	—	1	—	—	—	—	3	25,0
» I_2 ...	19	9	1	6	4	—	3	—	—	3	52,6
» I_3 ...	14	1	1	—	1	1	—	—	10	1	92,9
Line 4, I_1 ...	8	7	—	—	—	—	—	—	—	1	12,5
» I_2 ...	16	5	2	—	6	1	1	2	1	—	68,8
» I_3 ...	12	4	—	—	2	1	—	—	7	—	66,7
Line 9, I_1 ...	5	2	—	—	—	—	—	—	—	3	60,0
» I_2 ...	14	6	2	—	2	1	2	—	—	1	57,1
» I_3 ...	8	0	—	1	—	—	—	—	8	—	100,0

valents at the periphery of the plate are not stretched but more or less V-shaped.

In several cases the bivalents at I—M were arranged as a more or less clear ring (Fig. 19). In such cases the bivalents showed indications

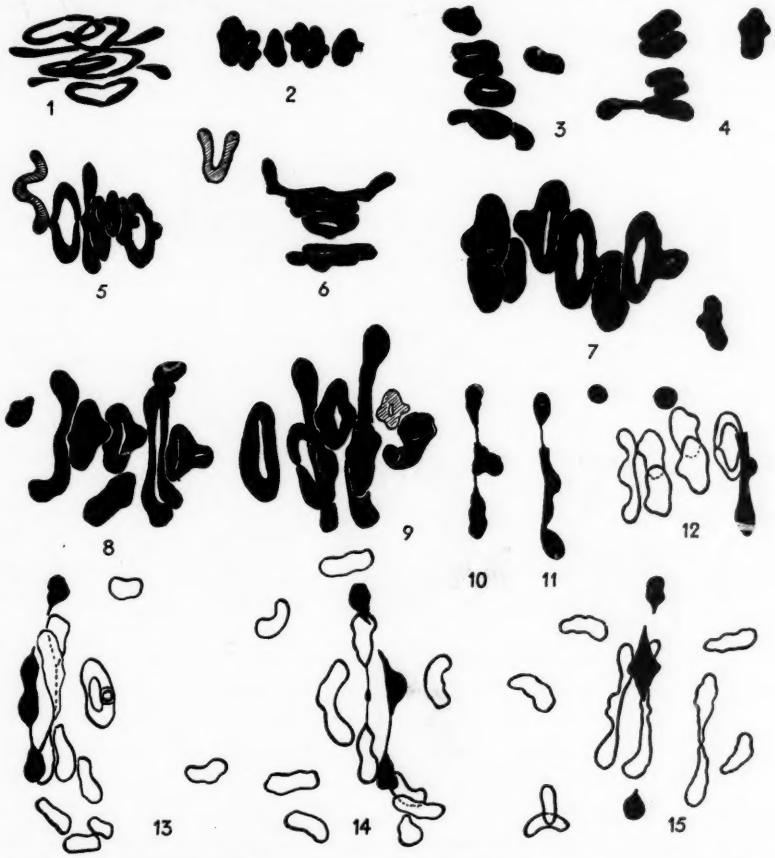

Fig. 1, I—M showing poor contraction. — Fig. 2, I—M, strong contraction. — Figs. 3—4, bad orientation of bivalents, one bivalent outside the plate. — Figs. 5—6, one rod bivalent with a peripheral position not stretched. — Figs. 7—8, 7 normal bivalents + 1 small bivalent. — Fig. 9, 6 normal bivalents, 1 abnormal bivalent + one smaller body, looking like a small bivalent. — Fig. 10, »lump» chromosome bivalent of normal appearance. — Figs. 11—15, cases of attenuation of the distal parts or fragmentation of the »lump» chromosome (in black). In Fig. 13, $4_{II} + 6_{I}$, in Fig. 14, $3_{II} + 8_{I}$, in Fig. 15, $3_{II} + 6_{I}$ + a lump chromosome bivalent, in which the distal parts are separated from the central part. — Figs. 7—9, × 3400; the other figures, × 2300.

of stickiness but probably a spindle abnormality is also involved. In some cases the stickiness at I—M was marked and led to a more or less pronounced agglutination of the chromosomes.

The remaining category of Table 6, »other irregularities», mainly includes cases of supernumerary bivalents and of fragmentation. In

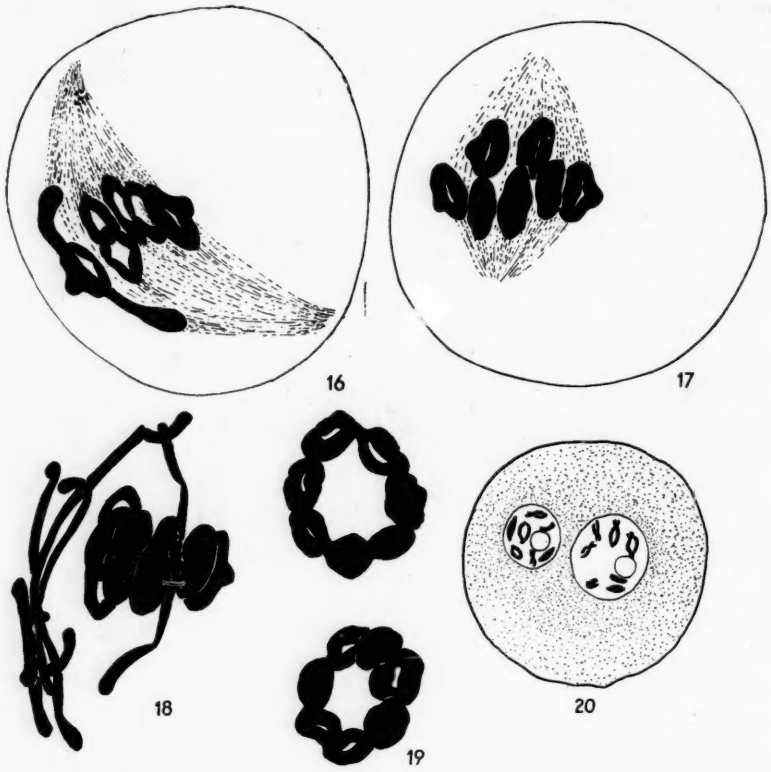

Fig. 16, I—M with 7_{II}, bent spindle with a lateral position. — Fig. 17, short and lateral spindle. — Fig. 18, I—M, two groups of about 7_{II} in the same p. m. c., one with very poor contraction, the other one with normal contraction. — Fig. 19, two I—M groups with a ring-shaped arrangement of the bivalents. — Fig. 20, a binucleate p. m. c. — Figs. 16—19, × 2850. — Fig. 20, × 950.

Figs. 7—8 there are seven normal bivalents and one additional body, which appears to be a small but otherwise typical bivalent. In Fig. 9 six of the bivalents are normal, the seventh seems to be broken, and close to this abnormal bivalent there is another body, apparently a

small bivalent. In all these cases it is a question of single deviating cells, the plants otherwise being normal.

A special bivalent (Fig. 10) seems to have a tendency to fragmentation, as may be seen from Figs. 11—15. In Fig. 11 one terminal section is only attached by a thin thread to the rest of the bivalent, and in Fig. 12 this section seems to be completely detached. Instead, two fragments of different size are present. In Figs. 13—14 the terminal sections of both chromosomes have only thin and partly broken connections with the central part, and in Fig. 15 there is a complete detachment. Figs. 13—15 also show a rather high frequency of univalents.

In some cases evidence of premeiotic irregularities was obtained. For instance, p. m. c.'s with two nuclei were observed (Fig. 20) and in Fig. 18 there are two groups of bivalents (approximately seven in each group) situated in the same cell. The two groups are strikingly dissimilar in respect of degree of chromosome contraction.

With regard to the degree of chromosome pairing significant line differences were observed (Table 5). The question may be raised whether line differences also occur in types and frequencies of the special meiotic abnormalities. In Table 6 (lower part) the data from the three best investigated lines are given, the number of individuals observed in each line being at least 5 per generation. Evidently there are no clear differences between the lines, possibly with the exception that in Line 3 (I_2) there is a more frequent occurrence of plants with strongly contracted chromosomes than in the other lines.

2. DIFFERENCES IN STAINING CAPACITY IN I_4.

In Table 1 the pairing values of 20 I_4 plants are also given. These 20 plants belong to two different groups, 186 and 187. These groups contain individuals representing a plus and a minus selection with regard to vigour. In I_3 the most vigorous as well as the least vigorous plants were isolated with bags in the usual way. Then the seeds obtained after isolation of 29 vigorous I_3 plants were mixed and sown in plot No. 186. In the same way the seeds from the isolation of 37 poor I_3 plants were mixed and sown in plot No. 187. Plots 186 and 187, both containing I_4 plants, were situated in the same field but separated by a distance of about 80 metres. The effect of the selection undertaken in I_3 on vigour in I_4 was obvious. The plants in plot 186, derived from the plus plants in I_3, were more vigorous than those in plot 187, representing the minus selection. This difference was verified

in the next generation, the kernels obtained after free intercrossing within the two groups being sown in a field trial. The offspring of the plus group gave a much higher yield than the minus group.

Of the 20 I_4 plants cytologically examined 9 belonged to the plus group, 186, and 11 to the minus group, 187. As may be seen from Table 1, there is no significant difference in degree of chromosome pairing between these groups. However, a striking difference was observed with regard to the staining capacity, the plus plants showing good staining in contrast to the minus plants. More precisely, the following data were obtained:

Plot No.	Staining		
	good	somewhat bad	bad
186 (+)	6	2	1
187 (—)	—	3	8

Though the numbers are small the significance of the difference is obvious. Thus, the reduced vigour of the plants in the minus material is correlated to a poor staining capacity.

The difference in staining was first observ[illegible]ides stained with crystal violet. New slides of 3 plants from No. [illegible] and 3 plants from No. 187 were made and stained according to the Feulgen technique. The result was just the same as before, i. e. good staining in the slides from the plus group and poor staining in the slides from the minus group.

The same identity of results from the two staining methods was also obtained with regard to 20 other plants also tested with the Feulgen method. These plants comprised 5 population plants (1 corresponding to I_1, 1 to I_2 and 3 to I_3) and 5 plants each of I_1, I_2 and I_3, these plants belonging to five different inbred lines. Each plant was represented by one slide, the total set of 20 slides being stained at the same time and under exactly the same conditions. As before, in the slides stained with crystal violet, only the slides of the I_3 plants were poorly stained, all the other slides being normal.

As in I_4 poor staining was correlated to poor vigour it might be expected that the I_3 and I_2 plants showing poor staining would also have less good vigour than the plants showing normal staining. However, the material available did not show any difference of this kind, plant height in both categories being equal.

IV. CORRELATIONS BETWEEN CHROMOSOME PAIRING, FERTILITY AND VIGOUR.

1. CHROMOSOME PAIRING AND FERTILITY.

The reduced amount of chromosome pairing occurring in many plants of the inbred generations may be expected to influence fertility. This was, indeed, found to be true, there being a positive correlation between chromosome pairing and pollen fertility in all three I-generations.

On account of the skewness of the distributions with regard to pollen fertility as well as degree of chromosome pairing it is more appropriate and also simpler to test the data obtained according to the χ^2-method than to calculate the coefficients of correlation. In I_1 the data obtained may simply be given as follows:

	Degree of chromosome pairing					
Per cent good pollen	0	—	13	—	14	n
90	12		10			22
100	8		21			29

Thus, among the plants having pairing values equal to 13 or higher the percentage of good pollen was generally in the class 90—100. Plants with lower pairing values than 13 had in most cases less than 90 per cent good pollen. A χ^2-test of the distributions 12 : 10 and 8 : 21 gave a χ^2 of 3,81 and a P of about 0,05.

In I_2 the larger number of individuals and the greater variation revealed a stronger and quite significant correlation, the following data being obtained:

	Degree of chromosome pairing					
Per cent good pollen	0	—	12	—	14	n
80	24		15			39
100	10		39			49

A χ^2-test of these distributions gives a χ^2 of 15,50 and a P smaller than 0,001.

Though less variable than the I_2, the I_3-generation also showed a significant correlation, as is evident from the following values:

Per cent good pollen	0 — 12	12 — 14	n
90	12	17	29
100	1	28	29

In this case χ^2 will be 12,0 and P smaller than 0,001.

As the correlation between chromosome pairing and pollen fertility is rather strong, it may be expected that the seed setting will also be influenced by the degree of chromosome pairing. The data on seed setting available only gave the number of seeds per isolated ear and for the degree of seed setting in the isolation bags the self-sterility factors are naturally of prime importance. Hence in I_1 and I_3 no correlation between chromosome pairing and seed setting could be established, but in the variable I_2 there was a significant correlation of this kind. This is evident from the following data:

Number of seeds per isolated ear	Degree of chromosome pairing													
	1 —	2 —	3	. . . .	7 —	8 —	9 —	10 —	11 —	12 —	13 —	14	n
0— 5	1	1		. . . .	2	2	3	3	1	12	6		31
5—65								1	10	13	2		26

If these series are compared with an analysis of variance, a v^2-value of 3,92 is obtained, which corresponds to a P slightly larger than 0,05. On account of the skewness of the first series it may be more appropriate to use a χ^2-test and in such a case the material may be grouped in the following way:

Number of seeds	Degree of chromosome pairing 0 — 11	11 — 14	n
0— 5	12	19	31
5—65	1	25	26

This gives a χ^2 of 9,76 and a P intermediate between 0,01 and 0,001. Thus it is evident that plants with good seed setting also have good chromosome pairing. The plants with poor seed setting in the isolation groups may have good or bad chromosome pairing. Bad seed setting may be a result of self-sterility factors or represent a true sterility caused by meiotic irregularities.

2. CHROMOSOME PAIRING AND VIGOUR.

As a measure of vigour plant height was used. Positive correlations between plant height and chromosome pairing were obtained in all three inbred generations, the following coefficients of correlation being obtained: + 0,30 (I_1), + 0,28 (I_2) and + 0,31 (I_3). The significance of these values were tested in the usual way according to the formula $t^2 = \frac{(n-2)r^2}{1-r^2}$. In all three cases the corresponding P values were intermediate between 0,05 and 0,01. Thus it may safely be concluded that there is a weak positive correlation between vigour and chromosome pairing, the vigorous plants on an average having better pairing than the weak plants. As an example of this correlation the data obtained for I_2 may be given (Table 7).

TABLE 7. *Correlation between chromosome pairing and vigour (I_2).*

Plant height (cm.)	Number of paired chromosome ends															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	n
60													1			1
70												1	1			2
80													1	2		3
90			1	1	—	—	—	—	2	1	1	—	4	3	1	14
100										1	2	3	2	6	3	17
110													1	9	2	12
120													2	2	2	6
130														2		2
140																
n:		1	1	—	—	—	—	2	2	3	4	11	25	8		57

V. DISCUSSION.

In the present material the degree of chromosome pairing in inbred plants and population plants was compared. In I_1 pairing was almost normal. A slight average decrease is probable, but the difference between population plants and inbred plants was not significant. In I_2, on the contrary, the effect of inbreeding on meiosis was very obvious and significant, the degree of chromosome pairing ranging from normal behaviour to almost complete asynapsis. In I_3, too, pairing was variable and less good than in the population plants.

Cytological investigations of inbred rye have previously been undertaken by LAMM (1936) and KAKHIDZE (1939). As mentioned in the introduction, LAMM studied material which had been inbred for about 8 generations, and KAKHIDZE also seems to have worked with stabilized

lines, inbred for several generations. In both cases an average reduction in pairing was observed. The present investigation shows that this reduction may be quite pronounced already in I_2 and that many plants in this generation show very marked meiotic disturbances. In exceptional cases a rather strong degree of asynapsis may also be found in population plants. Thus, in one individual fixed at the same time as I_1 the pairing value was as low as 6,0—6,5 (Table 1). In most population plants, however, the average number of paired chromosome-ends ranges from 12 to 14.

The decrease in chromosome pairing from I_1 to I_2 did not proceed further in I_3. On the contrary, the I_3 plants seem to have better chromosome pairing than I_2. A selection has probably occurred favouring plants with good pairing. The nature of this selection is not clear, but one possibility is revealed by the demonstration of a positive correlation between vigour and chromosome pairing. The plant percentage (the number of mature plants in per cent of the number of seeds sown) was lower in I_3 than in I_2, the values being 12,2 and 19,5 respectively. Thus, the rather low proportion of mature I_3 plants may represent a rather pronounced plus selection in vigour. This view is supported by the fact that the average number of tillers per plant was higher in I_3 than in I_2, the values being 9,0 and 4,3 respectively. An average difference between the years 1942 and 1943 may also be responsible, but the population plants fixed in these two years did not differ in average degree of chromosome pairing. Thus, it is more probable that the tendency to improved pairing in I_3 is due to selection than to environmental differences.

As to the causes of the reduced chromosome pairing in the inbred material, a first possibility might be that this reduction is only a secondary effect of the depression in vigour and the »physiological» disturbances in the inbred material. A general reduction in chromosome pairing is also met with in some aneuploids that have a disturbed physiology. This possibility is supported by the positive correlation between plant height and chromosome pairing which was found to occur in all three generations of inbreeding studied.

Besides coming under the general influence of the reduction in vigour that may occur the mode of meiosis in the inbred material is certainly also affected by genes with more or less specific effects. One such case has already been analysed by PRAKKEN (1943), viz. a case of asynapsis observed in an I_1 of the same rye variety as the present material. Offspring of this plant were studied in two generations and

evidence of monohybrid segregation was obtained, the recessives being asynaptic. This asynapsis, however, was not complete, a low proportion of bivalents being present. In nine plants examined the mean number of bivalents per cell ranged from 2,38 to 4,98. This would correspond to pairing values intermediate between 2,38 and 9,96. As may be seen from Table 1, several plants in our material, especially in I_2, show a similar degree of asynapsis. The rather continuous variation in pairing in I_2 indicates that several genes are involved. This, however, does not exclude the alternative that in special cases the poor pairing may be caused by single genes. Further evidence of a specific genotypical basis of the chromosome pairing is represented by the differentiation in this respect between lines and families and the positive correlation between mother plants and offspring. This has been discussed in detail above (pp. 490—495) and here it is sufficient to state that the total evidence demonstrates a significant differentiation between lines and families with regard to the degree of chromosome pairing. This differentiation is just starting in I_1—I_3 and may be expected to become clearer later on. At the same time, however, there will be a selection, favouring lines with good pairing. The present data revealed a positive correlation between degree of chromosome pairing and degree of fertility. Thus, bad pairing will contribute to the extinction of certain lines.

A differentiation in degree of pairing between different lines was also indicated in the material studied by LAMM and KAKHIDZE (l. c.), though the number of plants examined was not large enough to allow of definite conclusions. Both authors assume that this differentiation is the result of genetic segregation.

Inbreeding degeneration is generally assumed to be caused by homozygosity for recessive genes with more or less deleterious effects (cf. MÜNTZING, 1945). It is also assumed that these genes undergo Mendelian segregation, the only complication being linkage. From the present results it is clear that this segregation may be markedly influenced and disturbed by meiotic irregularities. These disturbances are not exceptional, but, at any rate in rye, a quite characteristic feature of the inbred material. Only the I_1 generation seems to have a normal cytology, already in I_2 the bad pairing and ensuing meiotic irregularities are very marked.

The genetic effects of such irregularities will be of three different kinds, of which the first is a disturbance of the Mendelian segregation by non-disjunction, elimination, etc. Secondly, meiotic irregularities will cause sterility and contribute to the extinction of such lines. Thirdly,

absolute homozygosity and constancy can only be obtained in lines with a stable meiosis. Selection will favour such lines, but the data so far available in rye indicate that a certain degree of meiotic instability will characterize most inbred lines. This may be expected to have genetic effects such as the segregation of new sublines in a material which, owing to prolonged inbreeding, should already be quite homozygous. Such results have been obtained in rye by MÜNTZING (unpublished data) and it may be suspected that the unexpected subline formation in maize observed by JONES (1939) may have similar causes.

A special peculiarity in the present material of rye is the poor staining capacity of the chromosomes in some plants. This was especially marked in I_3 and in one group of I_4 plants. As this poor staining was not only found with crystal violet but also with the Feulgen technique, it is evident that some plants have less nucleic acid than other plants. This may be the beginning of a line differentiation in regard to the quantity of nucleic acid which may become clearer in later generations of inbreeding. Theoretically, some individuals and lines may be expected to have an excess of nucleic acid, but no reliable evidence of such an excess is available. Several plants showed stickiness or agglutination of the chromosomes, but the possible relationship between these phenomena and the nucleic acid contents is obscure (cf. D'AMATO, 1948).

Reference should also be given to the peculiar chromosome blobs observed by PRAKKEN and MÜNTZING (1942, p. 469) in one inbred line of rye and in some F_1 plants from crosses between inbred lines. In these cases big spherical chromatin bodies were frequently detached from the ends of the so-called T-chromosomes. This phenomenon may possibly indicate an excess of nucleic acid in these plants.

The positive correlation between vigour and quantity of nucleic acid observed in I_4 is especially interesting, but at present it cannot be decided which is the cause and which is the effect. We are inclined to believe that the lack of nucleic acid in the minus group is just one symptom of the reduced vigour and the disturbed physiology caused by deleterious genes. It is not excluded, however, that the poor vigour is caused by a gene-controlled deficiency in the amount of nucleic acid.

The first alternative is supported by the observations of KOLLER (1946) concerning the contents of nucleic acid in the X-chromosome of the hamster. In this animal lack of nucleic acid is induced by environmental influences such as disease, lack of food and old age. In haploid rye the nucleic acid contents is lower than in diploid rye (LEVAN, 1942),

the chromosomes in the haploid, being differentiated spontaneously in the same way as in the diploids may be induced by cold »starvation». It seems probable that the low proportion of nucleic acid in the haploids is a consequence of their poor vigour and disturbed physiology.

Among the other deviations from a normal meiosis in the inbred plants the spindle abnormalities are predominating. Some of these abnormalities are directly visible such as the bent and lateral spindles, which are similar to those observed by SMITH (1934) in *Impatiens*. The poor or abnormal orientation of the bivalents at first metaphase is probably also a sign of defective spindles as well as the V-shaped rod bivalents situated at the periphery of the plates. The tendency to ring orientation of the bivalents in some plants indicates a hollow spindle. Though none of these phenomena have been analysed in detail it seems probable that the commencing genotypical differentiation in the first generations of inbreeding also influences the spindle formation in various ways. A gene-controlled spindle abnormality in maize has been described by CLARK (1940). In this case a recessive factor, induced by ultra-violet radiation, causes the fibres of the first meiotic spindle to diverge instead of converge at the polar regions of the spindle figure. In several cases of gene-controlled asynapsis the spindle is also abnormal (cf. BERGNER, CARTLEDGE and BLAKESLEE, 1934).

In our rye material single p. m. c.'s were repeatedly found to contain a supernumerary bivalent, which was generally smaller than the ordinary bivalents. The cause of this irregularity is obscure, but premeiotic irregularities may be responsible. Direct evidence of such irregularities was seen in the shape of p. m. c.'s with two nuclei or with the tetraploid number of chromosomes.

In the inbred plants the terminal portions of one of the rod bivalents were often seen to be far removed from the central part containing the chiasma, and in several cases a real rupture must have occurred. Most probably a special chromosome is involved in this tendency to fragmentation. This chromosome probably has a secondary constriction or some other weak point which, in inbred material, is liable to extension and breakage. The same chromosome has been observed by LAMM (1936, Figs. 7—8) and probably it is also related to the »lump» chromosome studied by MÜNTZING and PRAKKEN (1941, p. 289) in a case of segmental interchange.

The question may be raised whether the rather pronounced cytological effects of inbreeding which have been observed in rye represent a special case, or if cytological disturbances of this kind are of general

occurrence. Unfortunately, this problem has been very little studied in other groups of material. There is one exception, however, viz. the *Alopecurus* investigation by JOHNSSON (1944). This author has undertaken a detailed cytological investigation of inbred strains of *A. myosuroides*, and in the main there is a striking similarity between his observations and those made in rye. Thus, he finds all transitions from normal pairing to absolute asynapsis and he states that partial asynapsis is a very common phenomenon in some inbred families. He also finds cases of abnormally strong or weak chromosome contraction, stickiness, syncyte formation, and several other irregularities.

In view of this conformity between *Secale* and *Alopecurus* with regard to the cytological effects of inbreeding it is very likely that other organisms react in a similar way. If this is so, studies of the chromosomal behaviour are evidently indispensable in further work on effects of inbreeding and outbreeding. Especially desirable are studies of this kind in *Zea* and *Drosophila*.

SUMMARY.

(1) The degree of chromosome pairing in the first three inbred generations of *Secale cereale* was examined and compared with data from population plants. In I_1 pairing was almost normal but in I_2 and I_3 highly variable and on an average reduced.

(2) Differences in degree of pairing between inbred lines and families were established, and evidence of a positive correlation between pairing values of mother plants and offspring was obtained.

(3) Especially in I_3 and I_4 many plants showed a deficit of nucleic acid, the chromosomes being poorly stained. In I_4 this defect was correlated to vigour, the plants in a selected plus group showing better staining than the plants in a minus group.

(4) Positive correlations between chromosome pairing and male as well as female fertility were established. A positive correlation was also observed between plant height and degree of pairing.

(5) Several specific meiotic abnormalities were found to occur in the inbred plants, the most conspicuous ones being spindle abnormalities, premeiotic irregularities and cases of fragmentation.

(6) It is pointed out that the segregation of the genes responsible for the inbreeding degeneration may be markedly disturbed by the meiotic irregularities. These irregularities will also lead to sterility and contribute to the extinction of some lines. Absolute homozygosity and

constancy can only be obtained in lines with a stable meiosis. Selection will favour such lines, but a certain degree of meiotic instability will probably characterize most inbred lines.

(7) The similarity between the cytology of inbred material in *Secale* and *Alopecurus* indicates that the cytological reaction to inbreeding is of the same type in other organisms as well.

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ABSTRACTS — KURZE MITTEILUNGEN

GUNNAR ÖSTERGREN: Chromatin stains of Feulgen type involving other dyes than fuchsin.

As is well known, fuchsin sulphurous acid (Schiff's reagent) is in common use as a specific reagent for thymonucleic acid. The tissue or microtome sections to be investigated are first subjected to hydrolysis in normal HCl for variable times at 60° C and are after that put into the reagent, where a violet red colour develops. The process is known as the Feulgen reaction, and besides its use for the detection of thymonucleic acid it is also commonly employed as a highly specific cytological staining method for chromatin.

The present paper is a brief report of some investigations made by me to test whether it was possible also to use other dye-stuffs than fuchsin in this staining procedure. It is generally considered that in the Feulgen reaction the NH_2 groups of fuchsin react with sulphurous acid and with an aldehyde group liberated from the thymonucleic acid by the hydrolysis (cf. BAKER, 1942, pp. 21—22). As expected from this opinion I found that crystal violet, which is closely related to fuchsin but has $N(CH_3)_2$ groups instead of NH_2 groups, does not work in the Feulgen reaction. Many other dyes tested by me, however, were found really to act in the same manner as fuchsin when applied in this staining procedure. These dyes are all characterized by the fact that they contain at least one NH_2 group. The dyes successfully applied are: thionin, brilliant cresyl blue, neutral red, safranin, janus black and indoin blue.

The close relation to the Feulgen stain is especially clear in the case of thionin. Thionin, which in itself is deeply violet, is decolorized by the sulphurous acid fluid to give a faintly greenish-yellow solution. When slides of root tips (*Allium Cepa*) subjected to hydrolysis are put in this solution an intense bluish-green colour develops in the chromatin. The cytological picture is quite identical with that of a Feulgen staining, with the exception that the colour is a bluish-green instead of a violet-red. It should be remarked that a similar bluish-green coloration is obtained by adding formalin to the nearly colourless thionin sulphurous acid solution, from which it seems likely that such a coloration may be a characteristic of the aldehyde group. The colour is quite different from that of thionin itself, which demonstrates that it is not merely a case of reformation of thionin. In the case of most of the other dyes mentioned the sulphurous acid solution is not or but little decolorized, but the cytoplasmic stain obtained in these cases is usually easily washed out of the sections, whereas the chromatin stain firmly resists washing. In the case of janus black, however, the cytoplasmic stain seems to be more resistant, probably because of the phenol group present in this dye-stuff.

I suggest that these new cytological stains be called the thionin nucleal stain, the safranin nucleal stain, etc.

From the present results it can be predicted that many other dyes related to the above-mentioned ones may also be used in the same way. Thus, I do not have the slightest doubt that toluidine blue should also be applicable in such a staining procedure.

Finally, it should be mentioned that I have tested bismarck brown in preliminary experiments of this type. Notwithstanding the fact that this dye is

of a constitution quite different from the others mentioned here my experiments, although as yet not very decisive, indicate that it may be used in the same way. This dye also contains NH_2 groups.

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IVAR ELVERS: Permanent acetic smears.

In a previous communication (ELVERS, 1943) some experiments were recorded in which cellulose acetate had been used as a mounting medium for acetic smears. The method seemed promising, but attention was also drawn to some of its difficulties, especially the tendency of the medium to contract when the solvent evaporates.

In order to counteract this, further experiments were planned in which a plastifier was added to the cellulose acetate. As a preliminary step pure plastifiers were tried. The results with these turned out, in fact, to be in many respects so superior to those obtained with the cellulose acetate that the experiments with mixtures of the substances were postponed.

The preparations were made in the spring of 1945. As object were chiefly used pollen mother-cells of *Paris quadrifolia* in the stage of meiotic divisions. Acetic lacmoid was used for staining the chromosomes, this stain having less of a tendency than aceto-carmine or acetic orcein to blur the surrounding protoplasm.

The plastifiers used were *dibutyl phthalate* (obtained under the technical name of »palatinol») and *glyceryl triacetate* (technical name »triacetin»). These substances are thick, non-volatile fluids. Samples of them and of other substances used in my experiments have kindly been placed at my disposal by the WILHELM BECKER Co., Stockholm, through the courtesy of Messrs. B. RITOFF and H. JOHANSSON.

The mode of making the preparations is the simplest possible. The smear in the acetic solution is covered by a cover-glass, and strips of the plastifier are drawn along two opposite edges of the cover-glass in contact with the acetic solution. When the latter evaporates at the free edges, the plastifier is sucked in.

In some *Paris* preparations the mounting medium evidently did not always penetrate into the cells properly; these cells became badly shrunken. This was the case especially with the young pollen tetrads. In the proper division stages the tendency for this damage to be caused was far less. Some stray preparations of meristematic tissues, such as, for instance, conifer endosperms, also gave uniformly good results.

A recent re-examination of the preparations has shown that some more of them have deteriorated during the three years that have passed since their manufacture. In some cases the lacmoid has changed its colour into blue, at the same time also staining the protoplasm. This is probably due to the

reaction of the medium changing from acid to neutral. A number of preparations have also been more or less dried out. Either enough mounting medium had not been added, or else it had not been properly sucked in. (Two preparations had been sealed, the one with Canada balsam, the other with a water-soluble cellulose glue; both have been very well preserved.)

Anyway the examination has shown that about $^2/_3$—$^3/_4$ of the preparations still look very much the same as when they were new. It can be stated, thus, that the method provides an easy and reliable way of keeping many preparations in good condition for study for several weeks or months, and that it offers them a fair chance of being preserved for years.

As for a considerable period I shall probably not have time or opportunity to make any more extensive studies along this line of research, I should be very glad if my colleagues active in the field of cytology would find it worth their while to try the method, so that its usefulness for different objects and in the hands of different workers could be ascertained.

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YNGVE MELANDER: The use of dissolvable cover slips when making permanent squash preparations of chromosomes.

When examining chromosomes it is often useful to have them spread out, not concealing one another and lying at the same level. This is possible if, after or during a suitable fixation, the tissue can be converted in a very soft and tough condition with the chromosomes still unspoiled. Permanent squash methods in use all suffer from the disadvantage that it is difficult to remove the cover slip after the squashing without disturbing some part of the tissue film. It is also not easy to get just the right uniform pressure over the surface of the cover slip that will ensure an even and very thin film. This is particularly the case if the piece of tissue one intends to examine contains hard particles, which will crack the slide.

Working on planarian chromosomes, I found it almost impossible to remove the cover slip after applying a hard pressure, this owing to the fact that their sticky, mucous secretion when fixed causes it to adhere very hard to the slide. Searching for a way to avoid these disadvantages, I have found the following procedure to give very good results. It can be modified with advantage for other kinds of tissues than those of planarians. For instance, I have got very good preparations of mouse-testes.

Equipment. — Alcohol abs., 96 %, 50 %; acetic acid 45—50 %; acetone; distilled water; chromosome stain; mounting balsam dissolved in absolute alcohol; celluloid cover slips readily dissolvable in acetone but not in acetic acid, e. g. 20 × 30 × (0,05—0,2) mm. (celluloid meets these conditions); slides. The following accessories are useful but not absolutely necessary: a screw-vice — not too small — placed with its jaws horizontal; two iron plates with

ground surfaces, e. g. 77 × 26 × 10 mm; a tool consisting of a glass staff, flat at one end for the attachment of the living tissue to the slide and rounded at its other end for the preliminary spreading of the tissue after fixing.

Scheme of treatment. — (1) Attach living tissue to the slide by spreading it with a slight pressure. — (2) Put the slide a few seconds after attachment into a mixture of 60 % abs. alcohol + 20 % acetic acid + 20 % water. Let it remain there at least for 5 minutes. — (3) Transfer to 50 % acetic acid for 5 minutes . — (4) Allow any superfluous acetic acid to run off and place the dissolvable cover slip over the tissue and spread it out by pressing slightly over the cover slip, which ought not to be displaced. — (5) Apply a suitable, even pressure over the surface of the cover slip for about half a minute. I found it good to effect this pressure between two iron plates with ground surfaces and placed in a screw-vice. — (6) Dissolve the cover slip in acetone. It should be borne in mind that acetone is very volatile and that the slide will get dry within a few seconds when taken up from the liquid. — (7) Rinse in clean acetone. — (8) Rinse in $^1/_4$ acetone + $^3/_4$ water or 50 % acetic acid depending on the desired type of staining. — (9) Rinse in water and 50 % acetic acid, respectively. — (10) Staining. — (11) Transfer through, e. g., 50 % and 96 % alcohol to absolute alcohol. — (12) Mount in balsam dissolved in absolute alcohol.

Any common chromosome stain can be used. If one wants fairly permanent preparations by this method aceto-carmine with iron — 60 C° for 1—3 hours — Heidenhain's haematoxylin and Feulgen's reaction can be recommended. With a rapid method of staining the whole procedure will require less than half an hour. It is of course possible to use other fixatives suitable for squashes and smears and to macerate after fixing as is commonly done with plant tissues. It is seldom necessary to stick animal tissues to the slide by means of albumen.

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EEVA THERMAN-SUOMALAINEN: The structure of secondary constrictions.

Secondary constrictions have as a rule been represented as gaps in the chromosomes overbridged by a thin satellite thread. DARLINGTON (1937, p. 39), in fact, has defined all constrictions as being unspiralized regions in the chromosomes. During a previous study (SUOMALAINEN, 1947) on the chromosomal conditions in the genus *Polygonatum* it was found that the structure of the nucleolar constrictions in the somatic chromosomes did not agree with this definition. A closer examination of these constrictions in preparations, made with different fixing and staining methods, has revealed that they mostly appear as clear-cut, unstained gaps in the chromosomes. Sometimes the intervening region is stained, in which cases, however, it is not a thin thread, but is often seen as a broad zone, frequently equal in breadth to the main chromosome body (cf. RESENDE, 1940).

The SAT zone is similarly stained with the Feulgen method as with other

chromatin stains, from which it may be concluded that this zone has a thymonucleic acid charge, which, however, varies greatly from cell to cell.

The staining reactions of the SAT zones as well as their general appearance in *Polygonatum* bear strong evidence in favour of the opinion that they cannot be unspiralized threads but possess a spiral structure, this being, however, more labile than in other chromosome parts. Though the spiral structure is not actually visible in these regions, its existence is further supported by the behaviour of the chromosomes when, prior to fixation of the root tips, the plants are subjected to cold treatment. Cold seems to contract the main part of the chromosomes and the hiata created by the secondary constrictions almost proportionally, which may obviously be explained on the assumption that cold renders the spiralization of the SAT zones more compact, analogously to other chromosome parts.

In the various *Polygonatum* species cold treatment did not make visible any heterochromatic segments in the sense of DARLINGTON and LA COUR (1938, 1940). The secondary constrictions themselves in these plants might, however, be regarded as heterochromatic chromosome parts in the widest sense of this conception.

These questions will be considered more extensively in a later paper.

State Horticultural Institute, Piikkiö, Finland, March, 1948.

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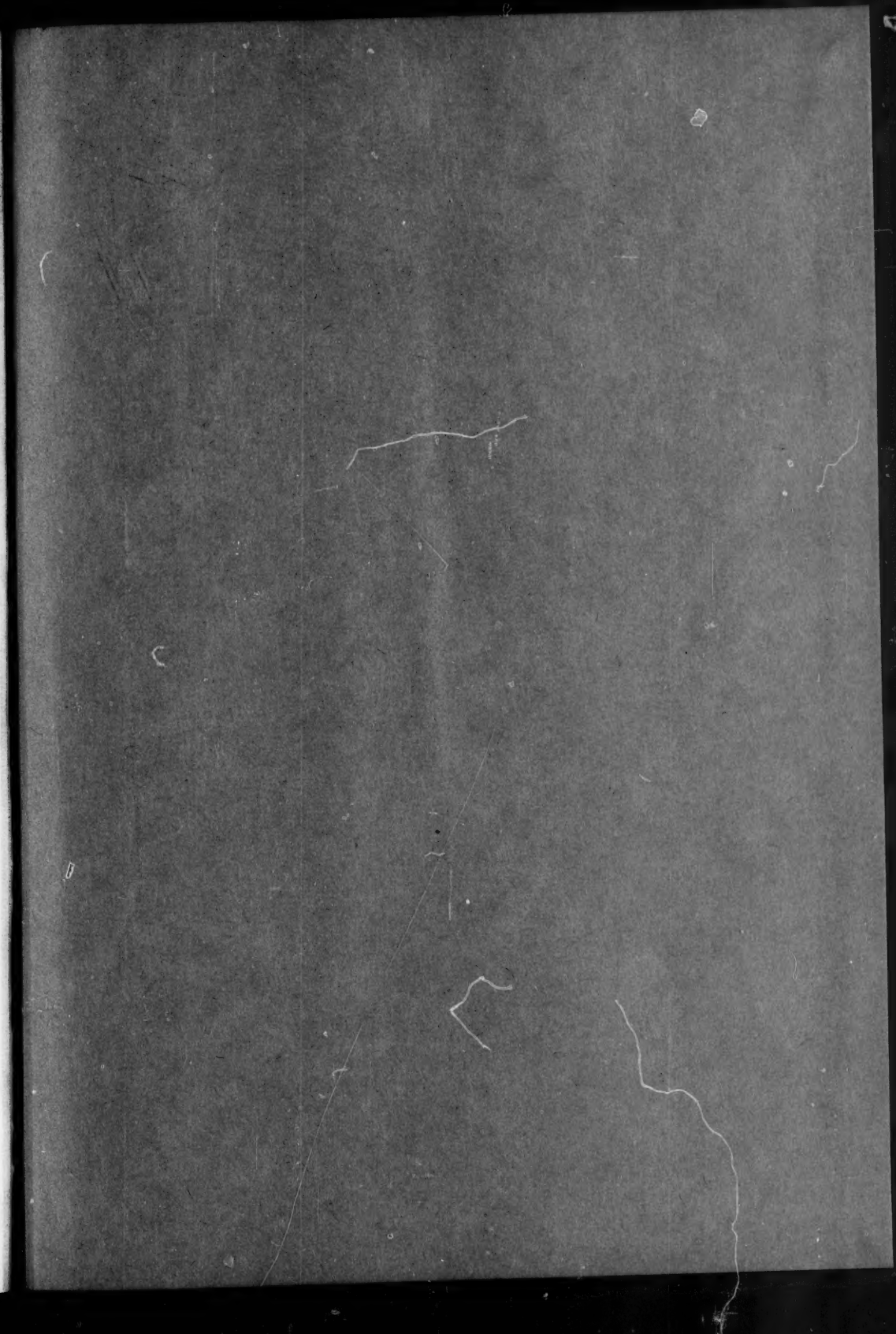
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